In vivo endotoxin synchronizes and suppresses clock gene expression in human peripheral blood leukocytes*

Beatrice Haimovich, PhD; Jacqueline Calvano, MS; Adrian D. Haimovich; Steve E. Calvano, PhD; Susette M. Coyle, MS, RN; Stephen F. Lowry, MD

Objectives: **The intravenous administration of a bolus dose of endotoxin to healthy human subjects triggers acute systemic inflammatory responses that include cytokine production and dynamic changes in gene expression in peripheral blood leukocytes. This study sought to determine the state of clock gene expression in human peripheral blood leukocytes, and leukocyte subpopulations, challenged with *in vivo* endotoxin at two circadian/diurnal phases of the clock.**

Design: **Clinical and laboratory investigation.**

Setting: **University-based research laboratory and clinical research center.**

Subjects: **Human volunteers.**

Interventions: **Human subjects were administered a standard dose of endotoxin (2 ng/kg) or saline at either 0900 or 2100 hrs. Blood samples were collected at selected time points pre- and postinfusion.**

Measurements and Main Results: **Clock gene expression was determined in human peripheral blood leukocytes, neutrophils, and monocytes by quantitative real-time polymerase chain reaction. The fold change for each gene was determined by use of the $2^{-\Delta\Delta C_T}$ method. We show that endotoxin causes profound suppression of circadian clock gene expression, clearly manifested in human peripheral blood leukocytes, neutrophils, and monocytes. *Clock, Cry1-2, Per3, CSNK1ε, Rora,* and *Rev-erb* gene expression were all reduced by 80% to 90% with the nadir between 3 and 6 hrs postinfusion. *Per1* and *Per2* reached an expression nadir between 13 and 17 hrs postinfusion. The levels of plasma interleukin-6 and tumor necrosis factor peaked and then returned to baseline within 6 hrs. In contrast, clock gene expression remained suppressed for up to 17 hrs irrespective of the phase of the clock at the time of the endotoxin challenge. Endotoxin did not perturb the melatonin secretory rhythm.**

Conclusions: **Circadian clock gene expression in peripheral blood leukocytes is dramatically altered and possibly uncoupled from the activity of the central clock during periods of acute systemic inflammation. The realignment of the central and peripheral clocks may constitute a previously unappreciated key factor affecting recovery from disease in humans. (Crit Care Med 2010; 38:751–758)**

KEY WORDS: endotoxin; circadian clock; peripheral blood leukocytes; melatonin

Numerous physiological activities in higher organisms are synchronized with the 24-hr rotation period of the Earth. The central "master" clock controlling behavioral circadian rhythms is located in the suprachiasmatic nucleus within the brain hypothalamus (1, 2). The molecular components of the circadian clock consist of transcription factors and proteins whose activity and/or availability cycle has a periodicity of approximately 24 hrs (3–5). Circadian oscillations of clock-associated genes have been identified in many tissues (6–9), highlighting the probable existence of multiple peripheral clocks. The mechanisms by which the master clock and the peripheral clocks interact are not completely understood.

Circadian rhythmicity and host immunity are closely interrelated. The number of circulating red blood cells, platelets, and all human peripheral blood mononucleated cell subsets exhibit significant diurnal variation (10, 11). Lange et al (12) reported that human monocytes obtained during normal sleep hours produce interleukin-12 (IL-12) in response to *ex vivo* endotoxin challenge, whereas monocytes obtained during the day produce IL-10. There is also evidence that intracellular IL-6 expression exhibits distinct diurnal pattern with increasing IL-6 levels during the night (13). These findings suggest that both immune cell number and function are subject to circadian regulation.

The core of the circadian clock is composed of two transcription factors, *Clock* and *Bmal1* (*Arntl; MOP3*), which form a heterodimeric complex. Analyses of mice with genetic alterations in *Bmal1* and *Clock* provided further insight into the relationship between immunity and the circadian clock. $Bmal1^{-/-}$ mice exhibit no locomotor rhythmicity and are significantly less active than normal mice (14). In addition, $Bmal1^{-/-}$ mice exhibit increased segmented neutrophils and platelets numbers and decreased B lymphocyte numbers (15). The daily rhythmic variability in white blood cell populations and the total number of white blood cells, red blood cells, and platelets were similarly reduced in mice that express a transcription-deficient mutant form of *Clock* (16).

Data suggest that the immune system is not only subject to circadian regula-

***See also p. 977.**

From the Department of Surgery, Division of Surgical Sciences, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School, New Brunswick, NJ.

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For information regarding this article, E-mail: haimovic@umdnj.edu or lowrysf@umdnj.edu

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tion, but may also provide inputs that alter the circadian activity of the clock(s) (17). It is also known that activities such as sleep, movement, and food intake that are normally regulated by the circadian clock are modified during periods of systemic inflammation (18, 19). Tumor necrosis factor alpha (TNFα), IL-1β, and IL-6 are among the prominent proinflammatory cytokines produced in humans during the acute period of systemic inflammation. Cavadini et al (20) reported that infusion of TNFα in mice triggered a significant reduction in locomotor activity, prolonged rest time, and impaired expression of several clock-related genes in hepatic tissue. The authors proposed that TNFα-induced behavioral changes were triggered, at least in part, through alterations in clock gene expression (20). These observations underscore the possibility that proinflammatory cytokines produced by activated immune cells have the capacity to reset the circadian clock in peripheral tissues during inflammation.

The status of circadian clock genes in humans during periods of systemic inflammation is largely undetermined. Okada et al (21) recently reported that the administration of endotoxin to rats impaired the expression of the clock genes *Per1, Per2,* and *DBP* in the liver, as well as *Per2* and *DBP* in the suprachiasmatic nucleus, with the expression nadir between 10 and 14 hrs postendotoxin challenge. The objective of this study was to determine whether clock gene expression is perturbed in human peripheral blood leukocytes (PBLs) during the acute period of systemic inflammation induced by an *in vivo* endotoxin (lipopolysaccharide) challenge (22, 23). Our data revealed that *in vivo* endotoxin causes profound and concurrent suppression of many clock genes in PBLs with a nadir between 3 and 6 hrs postinfusion. These observations identify endotoxin as a potent and acute entrainer of the circadian clock gene network in PBLs in humans.

MATERIALS AND METHODS

Study Population and Procedures. Male and female subjects (aged 18–29 yrs) were recruited by public advertisement to participate in a study approved by the Institutional Review Board of the Robert Wood Johnson Medical School. Subjects were administered a standard dose of endotoxin (2 ng/kg, National Institutes of Health Clinical Center Reference Endotoxin, CC-RE, Lot 2, Bethesda, MD) (24) or saline (0.9% sodium chloride). Additional information is provided in the Supplementary Materials (see Supplemental Digital Content 1, http://links.lww.com/CCM/A83).

Gene Expression Analyses in Peripheral Blood Leukocytes. Blood was drawn into Paxgene tubes (PreAnalytix). Total RNA was extracted as per PAXgene Blood RNA kit protocol (QIAGEN, Valencia, CA), quantified using Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA), and reverse-transcribed to cDNA using a high-capacity cDNA Archive kit (Applied Biosystems, Foster City, CA). Gene expression was analyzed in duplicates by quantitative real-time polymerase chain reaction using inventoried TaqMan gene expression assays (Applied Biosystems). A list of the gene expression assays is provided in the Supplementary Materials (see Supplemental Digital Content 1, http://links.lww.com/CCM/A83). The relative gene expression analysis was performed using the $2^{-\Delta\Delta C_T}$ method (25). The level of *β-2-microglobulin (B2M)* expression was used as an internal reference, because the expression of *B2M* is not affected by endotoxin administration *in vivo* (24, 26, 27). Data are expressed as fold change relative to time 0. For the sample at time 0, $\Delta\Delta C_T$ equals zero and 2^0 equals one, so that the fold change in gene expression relative to the control equals one (25).

Gene Expression Analyses in Leukocyte Cell Subpopulations. Detailed protocols can be found in the Supplement material (see Supplemental Digital Content 1, http://links.lww.com/CCM/A83).

Cluster Analysis. Details can be found in the Supplementary Materials (see Supplemental Digital Content 1, http://links.lww.com/CCM/A83). The data were clustered into a tree form using the standard hierarchial unweighted pair group method with one minus the crosscorrelation coefficient as a measure of similarity (28, 29). All clustering operations were carried out using MatLab 2008a.

Plasma determinations. TNFα, IL-6, and cortisol levels were determined as described (30, 31).

Melatonin. Plasma melatonin levels were determined using a direct melatonin radioimmunoassay kit (Rocky Mountains Diagnostics, Colorado Springs, CO). The assay range was 0–1000 pg/mL.

Statistics

Analysis of three groups or more was by one-way analysis of variance with Newman-Keuls posttest. Two groups were compared by unpaired Student's *t* test. The operations were carried out using Prism 4 software version 4.0b (GraphPad Software, Inc., LA Jolla, CA). *p* values $<.05$ were considered statistically significant.

RESULTS

Day Study. Subjects were administered endotoxin (n = 4) or saline (control; n = 2) at 0900 hrs (time 0). The relative gene expression values determined on the day before the challenge are shown in Supplementary Figure 1 (see Supplemental Digital Content 2). Of the 10 genes examined, only *Rora* and *Rev-erb* showed significant time-dependent expression differences before infusion ($p < .01$; one-way analysis of variance) with a peak at 0600 hrs (Supplementary Fig. 1, see Supplemental Digital Content 2, http://links.lww.com/CCM/A84).

The infusion of endotoxin triggered a significant decrease in *Clock, Per3, Cry1, Cry2, Rora, CSNK1ε*, and *Rev-erb* expression, reaching the nadir within 3–6 hrs (Fig. 1*A*) ($p \leq .0001$ for all genes; one-way analysis of variance). By 6 hrs postinfusion, the expression values of these seven aforementioned genes had decreased by 80% to 90% relative to baseline. *Bmal1* expression was not altered significantly postendotoxin infusion ($p > .05$, one-way analysis of variance). The expression values of all genes, with the exception of *Bmal1*, differed significantly over the interval between 0200 hrs (−7 hrs) preinfusion and 0200 hrs (17 hrs) postinfusion (Supplementary Table 1, see Supplemental Digital Content 3, http://links.lww.com/CCM/A85). These findings suggest that the expression levels of nine of the ten genes examined remained suppressed for at least 17 hrs postendotoxin challenge.

Plasma Cortisol and Proinflammatory Cytokines Levels in Endotoxin- and Saline-Challenged Subjects. Plasma cortisol levels in humans normally peak 3–4 hrs after the end of the sleep/darkness period (9, 32). Plasma concentrations of cortisol as well as the proinflammatory cytokines TNFα and IL-6 increase significantly in response to an endotoxin challenge (22, 31, 33). The anticipated increase in cortisol concentrations was detected in all endotoxin subjects by 1.5 hrs postchallenge. The cortisol concentration peaked between 3 and 6 hrs and returned to baseline levels at 24 hrs (Fig. 1*B*). As previously reported in this model system (22, 33), TNFα concentration peaked within 1–1.5 hrs postinfusion and returned to baseline by 3 hrs (Fig. 1*C*), whereas IL-6 concentration peaked within 2 hrs and returned to baseline within 6 hrs (Fig. 1*D*).

Nocturnal Study. We considered the circadian/diurnal phase at the time of endotoxin challenge as a possible influence on the expression of clock genes in endotoxin challenged PBLs. We therefore also studied volunteer subjects challenged with endotoxin at 2100 hrs (n = 3). Con-

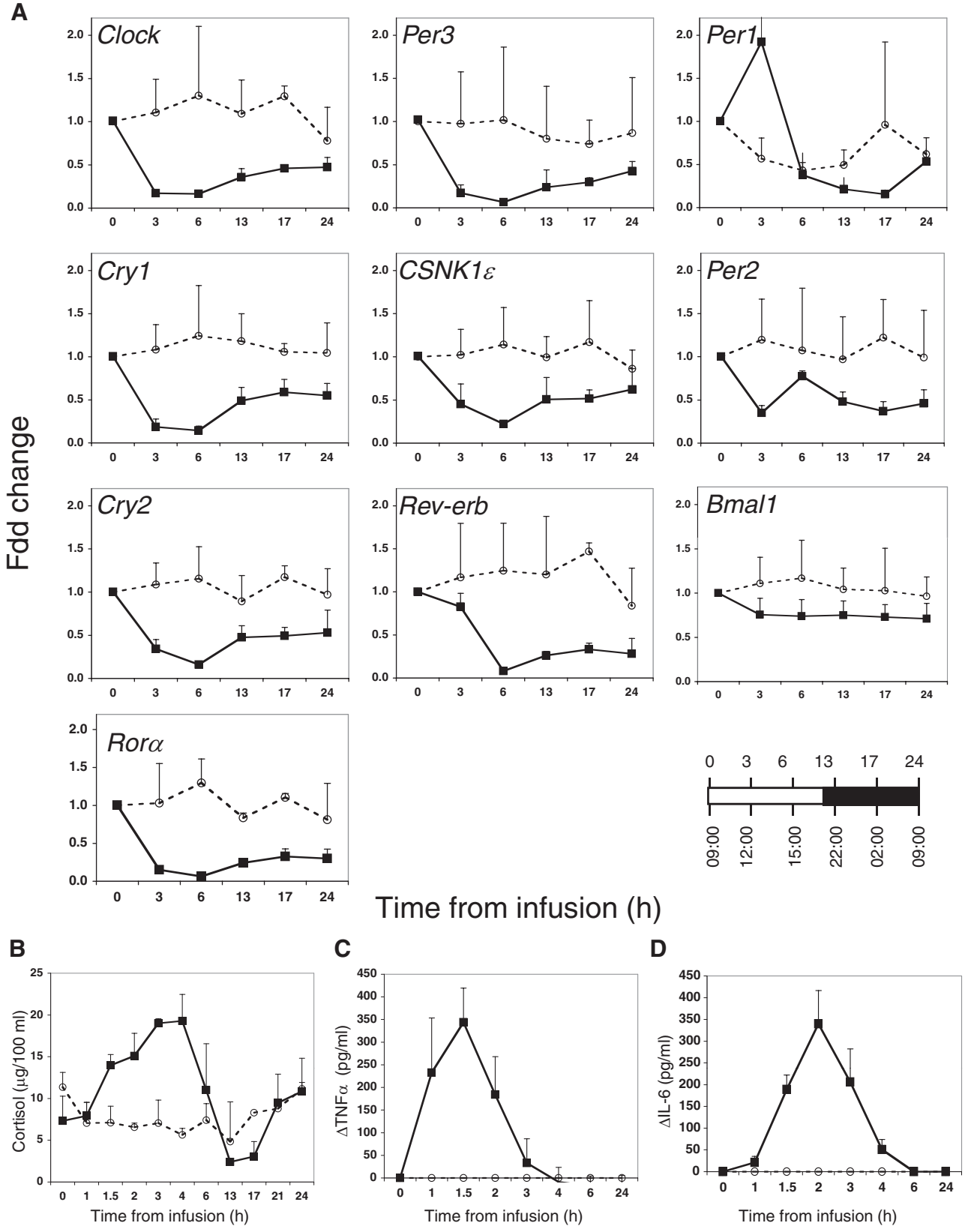

Figure 1. Clock gene expression in peripheral blood leukocytes obtained from human subjects challenged with endotoxin or saline at 0900 hrs. *A,* Blood was collected at the indicated time points postendotoxin (closed symbols; n = 4 subjects) or postsaline (open symbols; n = 2 subjects) infusion. Gene expression levels were analyzed by quantitative real-time polymerase chain reaction and are expressed as fold change relative to the 0900-hr infusion time taken to be time 0. The time from infusion relative to the time of day is illustrated in the lower right hand corner. Data are expressed as mean fold change ± SD. *B,* Plasma cortisol levels (n = 4) as a function of time postendotoxin were monitored by direct radioimmunoassay. *C,* Tumor necrosis factor alpha (n = 4) and (*D*) interleukin-6 (n = 4) levels were determined by enzyme-linked immunoassay.

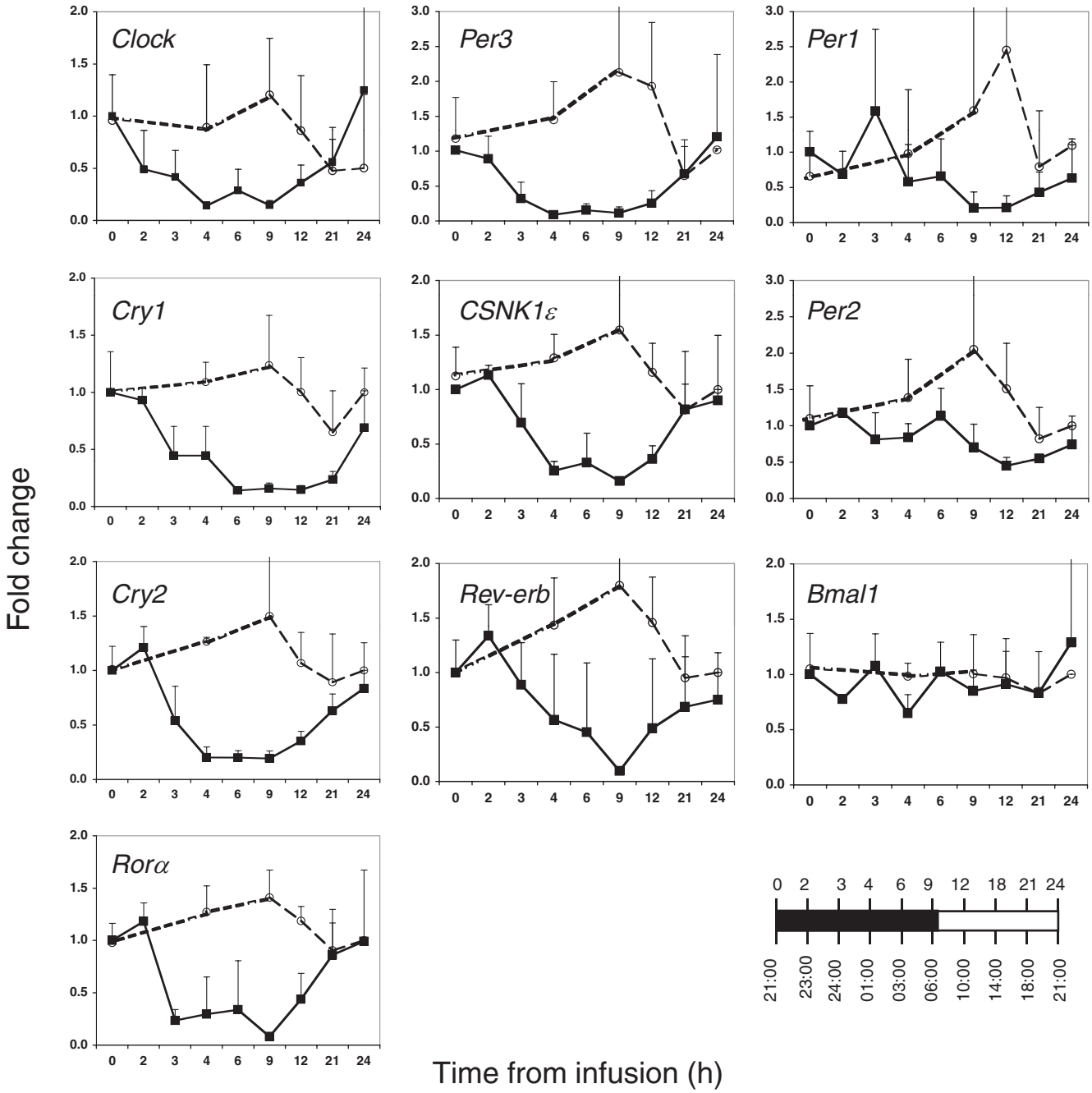

Figure 2. Clock gene expression in peripheral blood leukocytes obtained from human subjects challenged with endotoxin at 2100 hrs. Blood was collected at the indicated times before (open symbols) and after endotoxin infusion (closed symbols) (n = 3 subjects). Gene expression levels were analyzed by quantitative real-time polymerase chain reaction and are expressed as fold change relative to the 2100-hr infusion time taken to be time 0. The time from infusion relative to the time of day is illustrated in the lower right-hand corner.

sistent with the data presented in Figure 1*A* for the day subjects, the relative gene expression levels of all ten genes were close to or above baseline during the 24 hrs preinfusion (Fig. 2). The infusion of endotoxin at 2100 hrs triggered profound temporal changes in clock gene expression (Fig. 2). The changes were similar to those seen in subjects challenged with endotoxin at 0900 hrs. *Clock, Per3, Cry 1* and *Cry2, Rev-erb, Rora,* and *CSNK1ε* expression decreased by approximately 80% to 90% in response to the endotoxin challenge ($p < .001$) reaching their nadir between 4 and 9 hrs postinfusion. As was observed in the day subjects, the nadir in *Per1* expression occurred several hours after the nadir of most other genes. *Per2* and *Bmal1* were the least affected by endotoxin. These observations establish that endotoxin suppresses clock gene expression independent of the time of administration during the diurnal cycle.

Circadian Clock Gene Expression in Peripheral Blood Leukocytes and Leukocyte Subpopulations. Circadian clock gene expression was also compared among PBLs, monocyte, and neutrophil subpopulations (n = 3 for each). Blood

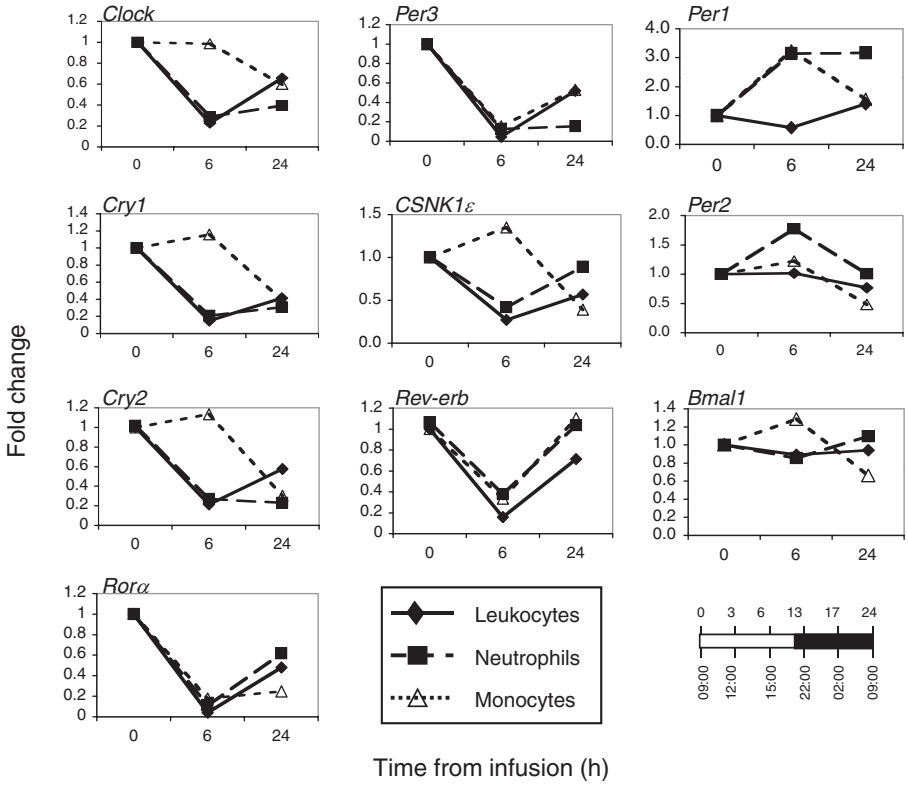

Figure 3. Clock gene expression in peripheral blood leukocytes, neutrophils, and monocytes obtained from human subjects challenged with endotoxin at 0900 hrs. Blood was collected at time 0, 6, and 24 hrs postendotoxin infusion. Total peripheral blood leukocyte, neutrophil, and monocyte subpopulations (n = 3 for each) were obtained before RNA isolation. Gene expression levels were analyzed by quantitative real-time polymerase chain reaction and are expressed as fold change relative to the 0900-hr infusion time taken to be time 0. The time from infusion relative to the time of day is illustrated in the lower right hand corner. The data shown are averages (n = 3) (all data sets are presented in Supplementary Fig. 2 [see Supplemental Digital Content 4, http://links.lww.com/CCM/A86]).

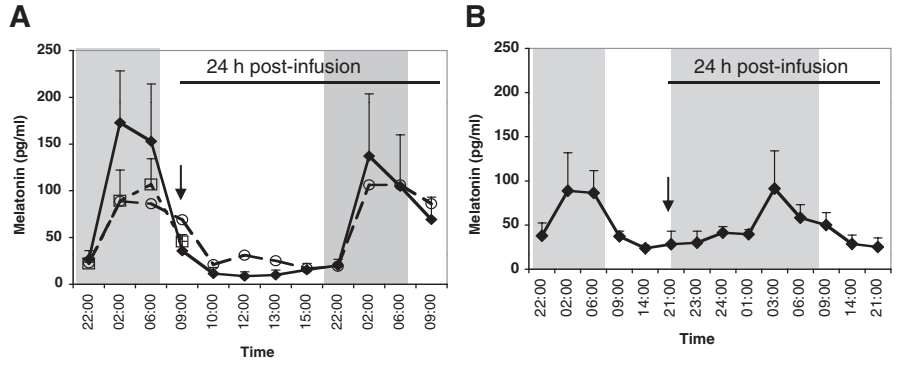

Figure 4. Endotoxin does not alter the melatonin secretory rhythm. *A,* Plasma melatonin concentrations were determined in blood samples obtained from control subjects (open squares; n = 8 subjects) at 2200 hrs, 0200 hrs, 0600 hrs, 0900 hrs, and 1000 hrs. Samples were also obtained at the indicated times of the day pre- and postsaline infusion (open circles; n = 2 subjects) and pre- and postendotoxin infusion (closed diamonds; n = 4 subjects). Arrow indicates the 0900-hr infusion time. *B,* Samples were obtained at the indicated times of the day pre- and postendotoxin infusion (closed diamonds; n = 4 subjects). Arrow indicates the 2100-hr infusion time.

was obtained before endotoxin infusion (time 0; 0900 hrs) and 6 and 24 hrs postinfusion. As shown in Figure 3 (additional data are presented in Supplementary Fig. 2 [see Supplemental Digital Content 4, http://links.lww.com/CCM/A86]), the expression patterns of *Clock, Cry 1–2, Rora, Per3, CSKN1ε, Rev-erb,* and *Bmal1* noted in PBLs were similar to those observed in neutrophils. The changes in *Clock, Cry1, Cry2,* and *CSKN1ε* expression observed in monocytes were time-delayed relative to those observed in neutrophils and PBLs. *Per3, Rora,* and *Rev-erb* expression was reduced in monocytes by 6 hrs postinfusion. *Per1* expression showed a great degree of variability within cell populations even when derived from a single donor (Fig. 3 and Supplementary Fig. 2 [see Supplemental Digital Content 4, http://links.lww.com/CCM/A86]). In contrast, as observed in PBLs, *Bmal1* expression was the least affected by endotoxin in neutrophils and monocytes. The data suggest that the temporal changes in clock gene expression observed in PBLs reflect changes that unfold in neutrophils and to a lesser degree in monocytes.

Endotoxin Does Not Affect the Melatonin Secretory Rhythm. The plasma melatonin levels in humans normally peak during the night (34, 35). Analyses of plasma melatonin levels before infusion, and post-endotoxin or -saline infusion, revealed the anticipated increase in melatonin in the late part of the night (Fig. 4). These observations suggest that endotoxin does not affect the apparent activity of the master clock during the acute period of systemic inflammation.

Circadian Clock Genes Exhibit Highly Related Responses to Endotoxin. To better define the relationship among circadian clock gene expression in endotoxin-challenged PBLs, the gene expression data presented in Figures 1 and 2 were clustered into a tree form (Supplementary Fig. 3*A–D* [see Supplemental Digital Content 5, http://links.lww.com/CCM/A87]). Clustering analyses of the combined day and night placebo data suggested correlations between *Per2* and *Bmal1*, and *Cry1* and *Cry2* (Supplementary Fig. 3*A* [see Supplemental Digital Content 5, http://links.lww.com/CCM/A87]). In contrast with the results observed for the placebo subjects, the clustering analyses identified four genes, *Clock, Per3, Cry1,* and *Cry2,* that had highly related responses to endotoxin in both the day (Supplementary Fig. 3*B* [see Supplemental Digital Content 5, http://links.lww.com/CCM/A87]) and night (Supplementary Fig. 3*C* [see Supplemental Digital Content 5], http://links.lww.com/CCM/A87) subjects. *Per 1, Per 2, Per 3,* and/or *Bmal1* showed a comparatively low degree of correlated expression in subjects administered endotoxin (Supplementary Fig. 3*B–D* [see Supplemental Digital Content 5, http://links.lww.com/CCM/A87]). Coclustering of the day and night expression data indicated that

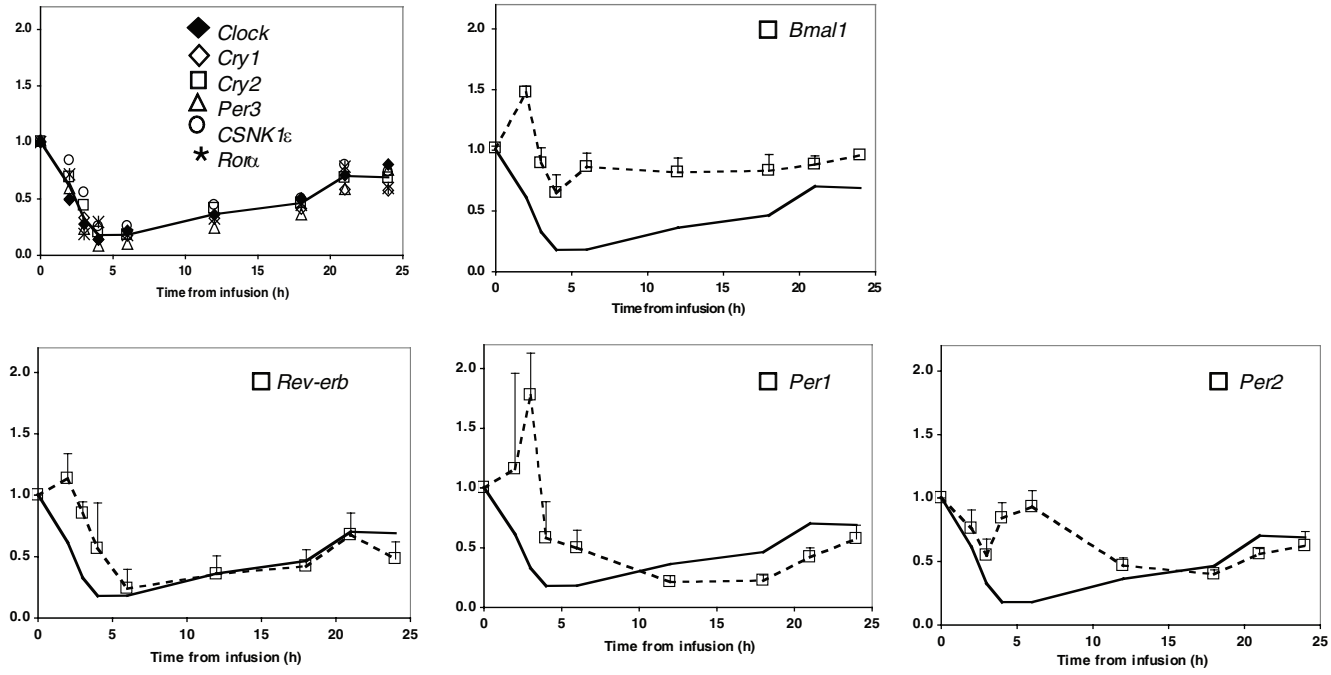

Figure 5. Clock gene expression in human peripheral blood leukocytes. The relative gene expression values for each gene postendotoxin infusion were averaged across all day (n = 3) and night subjects (n = 4). Where shown, bars represent SEM. The solid line that is featured in each panel was drawn based on the mean expression values of *Clock, Cry1, Cry2,* and *Per3,* which exhibited correlated expression in both the day and night subjects.

CSNK1ε and *Rora* patterns correlate with *Clock, Per3, Cry1,* and *Cry2* (Supplementary Fig. 3*D* [see Supplemental Digital Content 5, http://links.lww.com/CCM/A87]). When the day and night endotoxin subjects' data were analyzed as a group, the similar temporal decline in *Clock, Cry1, Cry2, Per3, CSNK1ε,* and *Rora* expression became apparent (Fig. 5). The solid line featured in each of the panels in Figure 5 represents the mean of *Clock, Cry1, Cry2,* and *Per3* expression values. *Bmal1, Rev-erb, Per1,* and *Per2* displayed distinct expression patterns compared with the common response pattern of the remaining six genes. The large number of clock genes exhibiting a similar endotoxin-induced response suggests that clock gene expression is both synchronized and suppressed during the acute period of systemic inflammation.

DISCUSSION

The Expression of Many Circadian Clock Genes Is Suppressed in Peripheral Blood Leukocytes During the Acute Period of Systemic Inflammation

We found that the expression of key genes implicated in the regulation of circadian clock function, including *Clock, Per3, Cry1* and *Cry2, Rora*, and *Rev-erb*, is decreased in PBLs by 80% to 90% within 3–6 hrs postendotoxin infusion. The *Clock/Bmal1* complex regulates the transcription of genes with an E-box promoter region, which include *Period (Per1, Per2, Per3*), *Cryptochrome* (*Cry1, Cry2*) and *Rev-erb* (36). Per/Cry protein complexes relocate from the cytosol to the nucleus, where they function as *Clock/Bmal1* repressors. *Rev-erba* and *Rora* bind to the promoter region of *Bmal1* to either suppress or enhance its expression, respectively (3). The repressor Per proteins are phosphorylated by casein kinase 1ε (CSNK1ε) (37). The phosphorylated proteins are ubiquitinated and targeted for degradation by the proteosome. Once the Per proteins are degraded, the derepressed *Clock/Bmal* complex reinitiates its activity cycle. The precipitous declines in expression of multiple genes that act at various points in the circadian clock network noted in this study provide a strong indication that clock activity in PBLs is severely impaired during the acute period of systemic inflammation.

Per1, Per2, Per3, and *Bmal1* gene oscillations have been observed in human peripheral blood mononucleated cells (PBMCs) as well as whole blood cells (9, 32, 38, 39). In one study (32), *Per1* and *Per2* expression in PBMCs peaked in the early hours of the day, whereas *Bmal1* peaked in the middle of the wake period. In another study, *Per2* and *Bmal1* cycled with a similar rhythm in PBMCs (39). Recently, the expression of ten circadian clock genes, including *Per 1–3*, *Cry1* and *Cry2*, *Clock,* and *Bmal1,* was examined in PBMCs (35). Of the ten genes examined, only *Per 1–3* showed rhythmic expression in most subjects with no significant acrophase differences among the three genes. The goal of the present study was to determine the fate of clock gene expression in PBL during the acute period of systemic inflammation. Hence, in contrast with the entrainment protocols used by others (9, 32, 35, 39), our subjects were not entrained by an extended sleep/wake schedule before or during the study phase. These differences may explain the limited similarity among *Per* gene expression patterns noted among our placebo subjects.

Prior studies revealed a limited coordination among clock gene expression patterns in PBMCs obtained from sleep/wake entrained normal human subjects and significant intersubject variability (32, 35, 39). In contrast, *Cry1* and *Cry2*, as well as *Clock, Per3, CSNK1ε, Rev-erb,* and *Rora*, exhibited correlated expression in PBLs obtained from endotoxin sub-

jects. The correlated expression patterns observed in PBLs, neutrophils, and monocytes after an endotoxin challenge suggest that endotoxin is a potent entrainer of the circadian clock network in circulating inflammatory cells.

Melatonin, Cortisol, and Circadian Clock Gene Expression in Peripheral Blood Leukocytes During the Acute Period of Systemic Inflammation

In humans, the activity of the central master clock is generally correlated with the secretion of melatonin in the middle to late part of the night (34, 35, 39, 40). We found that the melatonin rhythms remained intact in endotoxin-challenged subjects, peaking in the late part of the night. As previously reported (31), endotoxin triggered a surge in plasma cortisol levels with a peak between 3 and 4 hrs. It is interesting to note that while cortisol levels peaked, many clock genes reached their expression nadir. These data suggest that centrally regulated plasma melatonin and cortisol rhythms and PBL clock gene expression are independently regulated in response to endotoxin. These observations raise the possibility that the master clock and circadian clock gene expression in PBLs become misaligned during the acute period of systemic inflammation induced by endotoxin.

Okada et al (21) recently observed transient changes in clock gene expression in the suprachiasmatic nucleus and the liver of endotoxin-challenged rats. Endotoxin did not affect the rhythmicity of clock gene expression (21). Consistent with these observations, our data also indicate that endotoxin transiently suppresses clock gene expression in peripheral tissue(s). Furthermore, our data establish that in contrast with the significant perturbations in clock gene expression in PBLs, the rhythmicity of the master clock, determined based on plasma melatonin levels, appears to remain intact.

Despite our incomplete understanding of the mechanisms by which endotoxin influences circadian rhythmicity, a limited comparison between endotoxin and the two other well-characterized circadian entrainers, light and food, is possible. A short and abrupt period of bright light during the dark period is sufficient to reset the activity of the central clock in humans as determined by changes in core body temperature and plasma cortisol production (41). The magnitude of light-induced circadian phase resetting depends on the phase of the clock at the time of the input (41). In contrast, endotoxin appears to trigger similar changes in circadian gene expression in PBLs irrespective of the central clock phase. In addition, endotoxin disrupts the temporal relationship among plasma cortisol, melatonin, and circadian gene expression in PBLs. These observations suggest that the mechanism by which systemic endotoxin affects clock gene expression differs from the process by which light entrains the clock. The effect of endotoxin on the circadian activity may be more similar to that of feeding. In rodents, feeding during the subjective night or the subjective day alters the rhythmic expression of *Per1, Per2, Per3,* and *Cry1* in the liver within a few days. In contrast, the phase of *Per1* and *Per2* expression in the suprachiasmatic nucleus was not affected by food entrainment (42). Thus, both endotoxin and feeding appear to affect peripheral clock gene expression but not the activity of the central master clock.

Proinflammatory Cytokines and Clock Gene Expression in Peripheral Blood Leukocytes

Proinflammatory cytokines, which are released in the early stages of systemic inflammation, have been implicated in the regulation of circadian activity in mice. TNFα or IL-1β infusion suppressed the expression of several clock genes, including *Per2* and *Per3,* in mice livers by binding to the E-box motifs in their promoters (20). *Clock* and *Bmal1* expression were not affected by these cytokines (20). TNFα levels surge in response to endotoxin, reaching a zenith within 1.5–2 hrs post-challenge, whereas the expression of most clock genes examined in this study remained suppressed for up to 17 hrs. Thus, although TNFα and/or IL-1β are likely to contribute to the regulation of several clock genes, additional factors appear to regulate clock gene expression in human PBLs during the acute period of systemic inflammation.

Boivin et al (9) examined circadian clock gene expression in human PBMCs. Analysis of PBLs circumvents the need for cell-purification step(s), minimizes manipulation time, and hence is a practical approach for sample acquisition in the clinical setting. However, the use of a mixed cell population such as PBLs introduces some degree of uncertainty because the proportion of each immune cell type changes dynamically after endotoxin challenge, returning to baseline within 12 hrs posttreatment (22, 43, 44). To address this potential limitation, we compared clock gene expression among PBLs, monocytes, and neutrophils at select time points postinfusion. Our studies revealed that the changes in clock gene expression observed in PBLs were replicated in neutrophils. Interestingly, by 6 hrs postendotoxin infusion, several, but not all clock genes were also suppressed in monocytes. These findings establish that the changes in circadian clock gene expression observed in PBLs are primarily reflective of changes occurring in neutrophils and, to a lesser extent, in monocytes.

In conclusion, our study defines a severe misalignment of central circadian entrainment cues from peripheral clock gene expression in PBLs during acute systemic inflammation. Godin and Buchman (45) proposed that systemic inflammatory responses might trigger uncoupling between biological oscillators. Others have reported that the circadian rhythmicity of melatonin secretion is suppressed in severely ill patients (46, 47), whereas in rodents, absence of circadian cues during recovery from sepsis impairs survival (48). These findings add a new element to the potential link between circadian regulation and disease.

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