Rytmy w układzie odpornościowym

dr Magdalena Markowska
Czy są rytmy w układzie odpornościowym?

- TAK!

- Wykazano rytm krążących komórek odpornościowych, cytokin, wskaźników odporności nieswoistej

- Reakcje odpornościowe też wykazują cykliczność
Circadian rhythms in cell migration in vitro, and its effect on antigen-induced migration inhibition

Elizabeth Richens, K. A. Brown, A. J. Collins and P. A. Bacon

Fig. 1. Sequential measurements of (a) plasma cortisol, (b) total white cell numbers, (c) white cell migration area, and (d) the migration inhibition index (MI), from seven human volunteers over a 24-hr period. Vertical bars indicate standard error in the mean.
CIRCADIAN RHYTHMS IN CELL MIGRATION IN VITRO, AND ITS EFFECT ON ANTIGEN-INDUCED MIGRATION INHIBITION

ELIZABETH RICHENS, K. A. BROWN, A. J. COLLINS AND P. A. BACON

Fig. 2. Sequential measurements of (a) plasma corticosterone, (b) total white cell count and (c) white cell migration areas, from a group of fifteen rats, taken over a 24-hr period. Blood was collected by cardiac puncture. Vertical bars indicate standard error of the mean.
The time of day of antigen encounter influences the magnitude of the immune response

R. Pownall, P. A. Kabler & M. S. Knapp Chronotherapeutics Research Group, Renal Unit, City Hospital Nottingham

Table 1. The experiment design for the crossover and repeat studies

<table>
<thead>
<tr>
<th>Groups/time in crossover</th>
<th>Groups/time in repeat</th>
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<tbody>
<tr>
<td>A a B b C c</td>
<td>D E F</td>
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<tr>
<td>Initial challenge*</td>
<td></td>
</tr>
<tr>
<td>10.00 13.00 16.00</td>
<td>04.00 10.00 16.00</td>
</tr>
<tr>
<td>Re-challenge†</td>
<td></td>
</tr>
<tr>
<td>22.00 07.00 04.00</td>
<td>04.00 10.00 16.00</td>
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<tr>
<td>Change in clock time (hr)</td>
<td></td>
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<tr>
<td>12 18 13 12</td>
<td>0 0 0</td>
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</tbody>
</table>

* Day 9 (except E) when day 10.
† Day 21 (except E) when day 22.

Three crossover studies were performed pairing clock times 10.00 and 22.00 hr in A, 13.00 and 07.00 hr in B, and 16.00 and 04.00 in C. Ear challenges were carried out in the six groups of rats A, a, B, b, C and c at different clock times during the 24 hr period 9 or 21 days after initial sensitization to oxazolone. In groups D, E and F the first and second challenges were repeated at the same clock times.

Fig. 1. Mean ± s.e. increases in ear thickness (µm) 24 hr after oxazolone challenge in three crossover studies: (A) between 10.00 and 22.00 hr; (B) between 13.00 and 07.00 hr; and (C) between 16.00 and 04.00 hr. Within the blocks, the letters identify the groups, each of six rats, and the numbers indicate ear challenges 9 or 21 days after initial sensitization.

Light phase challenges

Dark phase challenges
The time of day of antigen encounter influences the magnitude of the immune response

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Fig. 2. Mean ± s.e. increase in ear thickness (μm) in groups of nine rats measured at 12 hr intervals after oxazolone challenge. The groups were challenged at 10.00 hr (●) or 22.00 hr (○) for the first time on light schedule 1. The light phase was then shifted by 12 hr, and the rats were rechallenged at the same clock times on light schedule 2.
Circadian rhythms in circulating T lymphocyte subtypes and plasma testosterone, total and free cortisol in five healthy men

F. A. LÉVI†, CHANTAL CANON*, Y. TOUITOU§, J. SULON§†, M. MECHKOUR†, EMILIE DEMEY PONSART†, J. P. TOUBOU†, J. M. VANNETZEL†, IRÈNE MOWZOWICZ‡, A. REINBERG‡ & G. MATHE* Institut de Cancérologie et d’Immunogénétique (CNRS UA04-1163), and SMST Hôpital Paul-Brousse, Villejuif, France, †Unité de Chronobiologie et Chronopharmacologie (CNRS UA51), Fondation A. de Rothschild, Paris, France, §Département de Biochimie, Faculté de Médecine Pitié-Salpêtrière, Paris, France, and ¶Département de Clinique et Pathologie Médicales, Université de Liège, Liège, Belgium

Fig. 1. Plexograms of circulating T, T helper and T suppressor-cytotoxic lymphocytes along the 24 h scale. An effect of sampling time was statistically validated by ANOVA (respectively $F = 6.8$, $F = 6.5$, $F = 4.2$; d.f. = 160; $P < 0.001$). A circadian rhythm with a period, $\tau \equiv 24$ h, was found for T and T helper lymphocytes by cosinor ($P < 0.001$). A rhythm, with $\tau \equiv 12$ h, was detected for T suppressor-cytotoxic lymphocytes ($P < 0.001$).
Circadian rhythms in circulating T lymphocyte subtypes and plasma testosterone, total and free cortisol in five healthy men

F. A. LÉVI*, CHANTAL CANON*, Y. TOUITOU*, J. SULON*, M. MECHKOUR*, EMILIE DEMEY PONSART†, J. P. TOUBOUĻ†, J. M. VANNETZEL†, IRÈNE MOWZOWICZŠ, A. REINBERG† & G. MATHE* *Institut de Cancérologie et d'Immunogénétique (CNRS UA04-1163), and SMST Hôpital Paul-Brousse, Villejuif, France; †Unité de Chronobiologie et Chronopharmacologie (CNRS UA581), Fondation A. de Rothschild, Paris, France; ‡Département de Biochimie, Faculté de Médecine Pitié-Salpêtrière, Paris, France; and §Département de Clinique et Pathologie Médicales, Université de Liège, Liège, Belgium

**Fig. 4. Acrophase chart of circulating total lymphocytes, T subsets and plasma cortisol and testosterone in diurnally active healthy subjects. Data from all five study months were pooled. The periods detected for each variable are indicated. Black dots indicate the location in time of the acrophase and the horizontal line its 95% confidence interval. When a 24 h rhythm was detected, the circadian acrophase is the only one shown. When a 12 h rhythm was detected alone (no 24 h rhythm), as was the case for T suppressor-cytotoxic cells, two acrophases are shown in the 24 h scale. The level of statistical significance of these circadian or circahrenidian rhythms was <0.001.**
RHYTHMS IN HUMAN BONE MARROW AND BLOOD CELLS

Rune Smaaland, Robert B. Sothern, Ole D. Laerum, and Jenny Foss Abrahamsen

Figure 5. Chronograms of seven different individuals in whom circadian variation in percentage of erythroid, myeloid, and total mononuclear human BM cells in S-phase. Similarities/variability in phasing (time of lowest and highest S-phase) is demonstrated between the seven different individuals as well as synchrony/variance in phasing between the three different cell populations in each individual series.
Twenty-Four-Hour Rhythms in Immune Responses in Rat Submaxillary Lymph Nodes and Spleen: Effect of Cyclosporine

Ana I. Esquifino, Laura Selgas, Agustín Arce, Valeria Della Maggiore, and Daniel P. Cardinali

Fig. 1. Twenty-four-hour changes in number of cells, LPS- and Con A-induced cell proliferation, and NK activity in rat submaxillary lymph nodes. Groups of 6–10 rats were injected with Freund’s complete adjuvant or its vehicle at 11:00 h. On the second day after injection, the rats were killed at six different times throughout the 24-h cycle and the submaxillary lymph nodes were dissected out and assayed as described under Materials and Methods. Shown are the means ± SEM. By a factorial ANOVA, immune

węży chłonne
Twenty-Four-Hour Rhythms in Immune Responses in Rat Submaxillary Lymph Nodes and Spleen: Effect of Cyclosporine

Ana I. Esquivio, Laura Sellos, Agustín Arce, Valeria Della Maggiore, and Daniel P. Cardinali

Fig. 2. Twenty-four-hour changes in number of cells, LPS- and Con A-induced cell proliferation, and NK activity in rat spleen. Groups of 6-10 rats were treated as in Fig. 1. Shown are the means ± SEM. By a factorial ANOVA, LPS- and Con A-induced cell proliferation in Freund’s adjuvant-injected rats was significantly higher than that in vehicle-treated rats ($p < .01$ and $p < .05$, respectively). A one-way ANOVA followed by a Student–Newman–Keuls’ test indicated time of day-related significant
Proliferacja splenocytów – wyniki własne

quail

chicken
Co generuje te rytmy?

- Zegar centralny SCN?
- Zegary peryferyczne umiejscowione w układzie odpornościowym?
- Rytmicznie wydzielane neurotransmitery i hormony?
- Czy rytm jest endogenny?
Role of sympathetic nervous system in the entrainment of circadian natural-killer cell function

Ryan W. Logan, Alvaro Arjona, Dipak K. Sarkar

Program of Endocrinology, Center of Alcohol Studies and Department of Animal Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ, USA

Fig. 1. The daily rhythm of NE content in the rat spleen. Male rats were sacrificed at 4-h intervals across a full day and spleens were collected for determination of NE content by HPLC-EC. Data are mean ± SEM, n = 5 per time point. *p < 0.05, significantly different from the lowest value (ZT19) per one-way ANOVA with Dunnett’s post hoc test.

Fig. 2. Effect of local splenic sympathectomy on spleen size and macroscopic appearance (A), spleen weight (B), and total number of lymphocytes (C) in NK cells, and splenic NE content (D) enriched at ZT7 and ZT19. Data are mean ± SEM, n = 4. *p < 0.05, significantly different from the other time point of the same group. *p < 0.01, significant difference between saline and guanethidine.
Role of sympathetic nervous system in the entrainment of circadian natural-killer cell function

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Fig. 4. Effect of local splenic sympathectomy on cytokines and cytolytic factors in NK cells. Protein levels of granzyme-B (A), perforin (B), IFN-γ (C), and TNF-α (D), in NK cells enriched at ZT7 and ZT19 from saline and guanethidine-treated spleens. Representative immunoblots, including actin (control), are shown. Data are mean ± SEM, n = 4. *p < 0.05, **p < 0.001, significant differences between time points within groups. *p < 0.01, significant difference between saline and guanethidine.
Cortisol and epinephrine control opposing circadian rhythms in T cell subsets

Stoyan Dimitrov, Christian Benedict, Dennis Heutling, Jürgen Westermann, Jan Born and Tanja Lange

Figure 7. Synopsis. Numbers of circulating T cell subpopulations show opposing rhythms with a nighttime peak (represented by naive CD4+ T cells; red cosine curve) or daytime peak (effector CD8+ T cells; blue cosine curve). Circadian rhythms peaking at night are controlled through the release of cortisol, which strongly increases during the early hours of daytime and via GR activation (with a delay of 3 hours) redistributes T cells from the circulation to bone marrow. Rhythms peaking during daytime are controlled by release of catecholamines, which is increased during daytime, and via β-AR activation leads to an immediate demargination of the cells from the vascular endothelium. Cortisol-sensitive T cells are characterized by high CXCR4 expression, whereas epinephrine-sensitive cells show highest CX3CR1 expression. The 2 chemokine receptors mark 2 distinct T cell populations with only few double-positive cells (contour plots for CXCR4 and CX3CR1 expression on CD4+ [top] and CD8+ T cells [bottom]). CXCR4 mediates homing of T cells to bone marrow with cortisol supporting this traffic by up-regulating CXCR4. Available data on CX3CR1 tempt to speculate that this molecule facilitates margination of T cells to vascular endothelium, with epinephrine inducing demargination by suppressing adhesive fractalkine signaling.
Fig. 1. Daily acrophase chart showing the effects of pinealectomy (Exp. I) and melatonin injections into the chickens pinealectomized (Exp. II) or with an intact pineal gland (Exp. III and IV) on the diurnal rhythm of peripheral granulocyte number. Different melatonin doses (MEL I, II, and III) or its solvent were injected for 4 weeks at the beginning of darkness (D, Exp. II and III) or 4 hr before the end of light (L, Exp. IV). Clear bar = light period; shaded bar = dark period; dots = acrophases; horizontal lines = 95% confidence limits.
Inflammation in the avian spleen: timing is everything

Kuller S Naidu, Louis W Morgan, Michael J Bailey

Figure 3 Quantitative RT-PCR analysis of cytokine gene expression in the spleen. Plotted open circles represent the mean ± SEM in each experimental group. The dashed line represents the fitted plot of cosine analysis utilizing linear harmonic regression. Values are represented as the number of transcript copies/1000 GAPDH transcripts. Asterisk labels indicate ZT time values every 3 hrs under LD 12:12 conditions; ZT0 = lights on; ZT12 = lights off. Open bar on the bottom of the x-axis indicates the light period, while crosshatched indicates darkness. TNFα mRNA achieves maximum levels at the dark-light transition (P<.001; P<.005; P<.005; P<.001), and IL-6 (P<.001; P<.001) mRNA each displayed a peak persistently after lights off at ZT12, while IL-1β (P<.005; P<.005; P<.001; P<.005) mRNA reached maximum values prior to midnight (ZT11). IL-2 and IL-12β mRNAs did not exhibit >2-fold change P<.005; P<.005; P<.005; P<.001; P<.005) mRNAs maintained robust circadian oscillations with maximal levels occurring at subjective midnight and subjective dawn, respectively. IL-2 (P<.005; P<.005; P<.005; P<.005) mRNA revealed that the mRNAs for each reached maximal values at early subjective day.
Inflammation in the avian spleen: timing is everything

Kallur S Naidu†, Louis W Morgan†, Michael J Bailey‡

Figure 5 Effects of acute melatonin and LPS administration upon cytokine induction in the spleen at midday versus midnight. Plotted values represent the mean ± SEM in each experimental group. Values are represented as the number of transcript copies/1000 GAPDH transcripts following the respective treatments, lipopolysaccharides (LSP), melatonin (Mel), or saline (Sal). ZT6 = midday; ZT18 = midnight. Melatonin was administered one hour prior to challenge with LPS or saline (ZT5). *Statistical significance is based on P < 0.05.
A two-clock model of circadian timing in the immune system of mammals

Un modèle à double horloge de chronométrage circadien dans le système immunitaire des mammifères

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Fig. 1. Role of melatonin in immune cells; mt: melatonin.

Fig. 2. Two-clock model of the regulation of circadian rhythms. One main clock is set into the nerve system, it is synchronized by light/dark regime and it is endogenous. Other clock is set into the immune system, it is synchronized by environmental factors or changes in the organism metabolism and it is exogenous. Both clock interact through hormonal messengers. SCN: suprachiasmatic nucleus.
Circadian Oscillations of Clock Genes, Cytolytic Factors, and Cytokines in Rat NK Cells
Alvaro Arjona and Dipak K. Sarkar

*J Immunol* 2005;174:7618-7624

Evidence supporting a circadian control of natural killer cell function
Alvaro Arjona, Dipak K. Sarkar

Endocrinology Program and Department of Animal Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ, USA

**FIGURE 2.** Canonical clock genes oscillate in enriched NK cells. Circadian rhythms in mRNA levels of *Per1* (A), *Per2* (B), *Bmal1* (C), *Clock* (D), and *Dbp* (E) detected in NK cells enriched from rat spleen at 6:00 a.m., 2:00 p.m., 6:00 p.m., 10:00 p.m., and 2:00 a.m. These time points correspond with ZT 3, 11, 15, 19, and 23, respectively. Total RNA from the enriched fraction was reverse transcribed to cDNA and then subjected to real-time PCR.

Reduction in mRNA levels was determined using a Student’s t-test with Bonferroni’s correction for multiple comparisons.
Inflammation in the avian spleen: timing is everything

Kallur S Naidu, Louis W Morgan, Michael J Bailey

Figure 1 Quantitative RT-PCR analysis of circadian clock gene expression in the spleen: Plotted open circles represent the mean ± SEM in each experimental group. The dashed line represents the fitted plot of cosine analysis utilizing linear harmonic regression. Values are represented as the number of transcript copies/1000 Gapdh transcripts. Abscisca labels indicate ZT time values every 3 hrs under LD 12:12 conditions; ZT0 = lights on; ZT12 = lights off. Open bar on the bottom of the x-axis indicates the light period, while crossed hatches indicates darkness. The cyp1a and per genes exhibit daily oscillations >3.5 fold in amplitude with higher abundances occurring during the late night for cyp1a (P<0.01; PERIOD<0.05) and per2 (P<0.05), and per3 (P<0.01); PERIOD<0.05). Clock and the bmal1 also express a daily pattern of rhythmicity in the spleen. Clock mRNA attained maximal abundance during the early night (P<0.01; PERIOD<0.05) while bmal1 is highest during the late night to early day period (P<0.05; PERIOD<0.01), showing increased expression during the nighttime (P<0.01; PERIOD<0.05), however it was less robust as other clock genes nor in excess of a 2.5 fold rhythm.

Figure 2 Quantitative RT-PCR analysis of circadian clock gene expression in the spleen: Plotted dark circles represent the mean ± SEM in each experimental group. The dashed line represents the fitted plot of cosine analysis utilizing linear harmonic regression. Values are represented as the number of transcript copies/1000 Gapdh transcripts. Abscisca labels indicate ZT time values every 3 hrs under LD 12:12 conditions; crossed hatches indicates darkness. Clock genes display robust 24 hr oscillations with maximal amplitudes occurring in the late subjective night and early day for cyp1a (P<0.01; PERIOD<0.05), cyp2a (P<0.05) and per2 (P<0.05; PERIOD<0.01) and per3 (P<0.05; PERIOD<0.01). Clock (P<0.01; PERIOD<0.005; PERIOD<0.05) shows increased expression during the night time (P<0.05; PERIOD<0.01), however it was less robust as other clock genes nor in excess of a 3 fold rhythm.
Figure 1. Circadian variation of clock gene mRNA transcripts in human PBMCs. Transcript expression of hPer1, hPer2, hPer3, and hDec1 for each subject is shown with a solid line and the dual-harmonic regression with a dotted line. The y-axis represents the relative intensity of mRNA expression. The value of the lowest mRNA expression is designated 1, and the levels of mRNA expression at all other time points are calibrated to this value. Error bars indicate the standard deviation on the basis of the mRNA samples assayed in triplicates. The x-axis indicates the time, in hours, under the constant-routine procedure. In the melatonin (MLT) panel, the y-axis represents plasma melatonin concentration (picograms per milligram) in each subject. For the purposes of illustration, times where subjects are habitually asleep are projected as open rectangles (□).
A circadian clock in macrophages controls inflammatory immune responses

Maren Keller<sup>a,1</sup>, Joannine Mazuch<sup>a,1</sup>, Ute Abraham<sup>b</sup>, Gina D. Eom<sup>b</sup>, Erik D. Herzog<sup>b</sup>, Hans-Dieter Volk<sup>c</sup>, Achim Kramer<sup>a,2</sup>, and Bert Maier<sup>a</sup>

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Edited by Joseph S. Takahashi, University of Texas Southwestern Medical Center, Dallas, TX, and approved October 20, 2009 (received for review June 12, 2009)

Fig. 1. Fully competent circadian clocks in tissues and cells of the immune system. (Left) Circadian clock genes Per2 (filled circles) and Rev-Erbα (open circles) are rhythmically expressed in murine spleen cells, lymph nodes, and peritoneal macrophages. Tissues and cells were harvested at regular intervals over the course of the first 2 days after transfer of the mice from a LD cycle to DD. Gray and black bars refer to the previous light and dark periods, respectively. CT 9 corresponds to the time in DD when the light would have turned on in the prior LD cycle. Transcript levels were analyzed by using quantitative RT-PCR. Displayed are the means ± SEM. (spleen: n = 3–6; lymph nodes: n = 3–4 except for n = 2 at CT 8/day 2; macrophages: n = 3–6) normalized to nonoscillating Gapdh expression levels. (Right) Autonomous clock gene oscillations in macrophages and lymph nodes. Relative mRNA abundance is shown as fold change (FC) compared with the CT 0 time point. (C) Relative bioluminescence of TNF-α, IL-6, and IL-12 in macrophages and lymph nodes. Relative bioluminescence is shown as fold change (FC) compared with the CT 0 time point.

Fig. 2. Circadian cytokine secretion upon challenge with bacterial endotoxin. (A) Spleens from C57BL/6 mice transferred in DD were harvested at regular 4-h intervals. After stimulation with LPS, TNF-α (Left) and IL-6 (Right) secretion was determined by ELISA. Gray and black bars refer to the previous light and dark periods, respectively. Presented are the means ± SEM (n = 4–5).
A circadian clock in macrophages controls inflammatory immune responses

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Fig. 3. A macrophage intrinsic clockwork regulates circadian TNF-\(\alpha\) and IL-6 secretion upon LPS stimulation. (A) Circadian modulation of LPS-induced cytokine response is independent of systemic cortisol. Spleens from adrenalectomized C57BL/6 mice were harvested and analyzed as described in Fig. 2. TNF-\(\alpha\) and IL-6 cytokine secretion per macrophage was determined via ELISA by taking the absolute number of monocytes/macrophages of the spleen into account (see also Figs. S2 and S4A and Materials and Methods). Circadian
A circadian clock in macrophages controls inflammatory immune responses

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Fig. 5. Circadian transcription of genes contributing to LPS response. (A) Gene regulatory network forming the LPS-triggered cytokine response. Dark gray boxes indicate circadian transcriptional regulation of the respective gene (P < 0.05). Arrows indicate molecular interaction of genes involved in LPS response generation. For detailed information about circadian transcripts in this network, see Table S2. (B) Selected circadian transcriptional profiles of genes participating in LPS-triggered signaling cascade. Individual datasets from microarray analysis were plotted (filled circles) together with data obtained by a quantitative RT-PCR assay of the same samples (open circles, means ± SEM, n = 4, except of times CT 24 and 28, n = 3). Statistical analysis for qPCR data and chip data were performed with CircWave and CircWaveBatch, respectively (microarray: P = 0.0001: Jun, Adam17, Cd180; P < 0.01: IkBa, MD-1, Elavl1, Fos, Erk1; qPCR: P < 0.0001: Adam17, Elavl1, Cd180, Erk1, MD-1; P = 0.01: IkBa; P = 0.05: Jun).
Fig. 4. Mean counts of circulating leukocytes, as percent of individual mean, according to sampling time in rats kept under LD12:12, with light from 0800 to 2000 (●), or under LL (△) or DD (■) (experiment I). In LD12:12, a group 24-h rhythm was detected with an amplitude of 18% and a maximum near 1100 (P < 0.001). In LL, the rhythm persisted with a similar amplitude (17%) and time of maximum (1200; P < 0.001). Its mean individual peak-trough difference (dPT; ±SE) was reduced compared with LD12:12 (42 ± 5 vs. 62 ± 7%, respectively, P = 0.04). In DD, its rhythm had similar amplitudes and times of maximum compared with LD12:12 and a restored mean dPT of 70 ± 5%.
Persistent twenty-four hour changes in liver and bone marrow despite suprachiasmatic nuclei ablation in mice

Elisabeth Filipski, Verduin M. King, Marie-Christine Etienne, XiaoMei Li, Bruno Claustrat, Teresa G. Granda, Gerard Milano, Michael H. Hastings and Francis Lévi


Fig. 6. Nucleated cell count in the bone marrow of sham-operated and lesioned mice as a function of sampling time. Each point represents mean ± SE of 5–6 sham-operated mice (●) or 8–9 lesioned mice (○). Open horizontal bars, light span; filled horizontal bars, dark span.

Fig. 7. Proportion of cells in G1 (●), S (○), and G2-M (▲) in the bone marrow of sham-operated mice (●) and lesioned mice (○) as a function of sampling time. Each point represents mean ± SE of 5–6 sham-operated mice or 8–9 lesioned mice. Open horizontal bars, light span; filled horizontal bars, dark span.
Fig. 3. Effect of local splenic sympathectomy on daily clock gene expression. Protein levels of PER2 (A) and BMAL1 (B) in NK cells enriched at ZT7 and ZT19 from saline and guanethidine-treated spleens. Representative immunoblots, including actin (control), are shown. Levels of mRNA Per2 (C) and Bmal1 (D) in whole-spleen at ZT7 and ZT19. Data are mean ± SEM, n = 4. *p < 0.05, **p < 0.001, significant differences between time points within groups. *p < 0.01 significant difference between saline and guanethidine.
The Circadian Clock *Period 2* Gene Regulates Gamma Interferon Production of NK Cells in Host Response to Lipopolysaccharide-Induced Endotoxic Shock

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Received 21 February 2006/Returned for modification 24 April 2006/Accepted 17 May 2006

FIG. 1. Survival of wild-type and *Per2<sup>−/−</sup>* mice injected with LPS. (A) Ten mice per group of each strain were kept under a cycle of 12 h dark and 12 h of light for 2 weeks and were challenged with 25 mg/kg of LPS at 9 a.m., 2 p.m., 9 p.m., or 2 a.m. The mice were observed for 2 h continuously for up to 96 h, at which point the rate of survival was calculated. All surviving mice eventually recovered from shock induced by LPS injection. (B) Kinetics of LPS-induced shock/death in wild-type mice and *Per2<sup>−/−</sup>* mice following LPS injection at 2 p.m. Survival rates were calculated based on results for 10 mice per strain 48 h after LPS injection.

FIG. 2. Serum cytokine levels after LPS injection in vivo. Wild-type (WT) and *Per2<sup>−/−</sup>* mice were injected i.p. with 25 mg/kg LPS. Five mice were sacrificed at each time point (1, 3, 6, and 12 h) after LPS challenge, and serum cytokine levels were measured from 100 μl serum by ELISA. ANOVA was used for multigroup comparisons, followed by Tukey's multiple-comparison test if ANOVA showed a significant difference. T, time (in hours).
Evidence supporting a circadian control of natural killer cell function

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Received 6 September 2005; revised in revised form 9 October 2005; accepted 11 October 2005

Available online 23 November 2005

Fig. 4. Protein levels of granzyme B (A), perforin (B), IFN-γ (C), and TNF-α (D) in mock-transfected, control siRNA-transfected, and Per2 siRNA-transfected RKN16 cells. Representative immunoblots, including actin (loading control), are shown. Data are means ± SEM of three independent experiments. a, significantly different (p < .05) from mock-transfected cells. b, significantly different (p < .05) from control siRNA-transfected cells.
The nuclear receptor REV-ERBα mediates circadian regulation of innate immunity through selective regulation of inflammatory cytokines

Julie E. Gibbons, John Blakley, Stephen Beesley, Laura Matthews, Karen D. Simpson, Susan H. Boyce, Stuart N. Farrow, Kathryn J. Else, Dave Singh, David W. Ray, and Andrew S. I. Loudon

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Fig. 1. Circadian gating of murine cytokine responses to LPS. (A) Serum cytokines were quantified 4 h after i.p. LPS administration at either CT0 or CT12. IL-6, IL-12(p40), CCL5, CXCL1, and CCL2 (but not TNF-α) showed significantly higher levels after CT12 challenge vs. CT0 (n = 7-9, two-way ANOVA, post hoc Bonferroni). (B) mRNA was isolated from PECs harvested 30 min after LPS treatment at either CT0 or CT12. Levels of cytokine mRNA were quantified (relative to β-actin) and are presented in relation to expression levels in PECs harvested at CT0 from vehicle-treated mice (n = 6-7, t-test).
The nuclear receptor REV-ERBα mediates circadian regulation of innate immunity through selective regulation of inflammatory cytokines

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**The nuclear receptor REV-ERBα mediates circadian regulation of innate immunity through selective regulation of inflammatory cytokines**

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**leukocyty z jamy otrzewnej**
The nuclear receptor REV-ERBα mediates circadian regulation of innate immunity through selective regulation of inflammatory cytokines

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Fig. 4. Circadian gating in human cells. (A) In human MDMs, il6 mRNA response to LPS peaks 16 h after serum synchronization (values are mean ± SD, n = 4). (B) In response to LPS, IL-6 release (but not IL-8) is inhibited by the REV-ERB ligand GSK4112 in both MDMs (n = 3) and primary alveolar macrophages (n = 18). Cells were incubated with LPS and GSK4112 for 16 h before harvest (one-way ANOVA, post hoc Bonferroni). (C) IL-6 protein (but not IL-8) expression by THP-1 cells in response to LPS is significantly attenuated by application of GSK4112 (one-way ANOVA, post hoc Bonferroni). (D) il6 mRNA expression after LPS application is reduced by both GSK4112 and hemin in THP-1 cells (n = 3, one-way ANOVA, post hoc Bonferroni); transcript abundance is reported relative to LPS alone. (E) shRNA knockdown of rev-erbα in THP-1 cells increases il6 (but not il8) mRNA after LPS challenge compared with controls (n = 3, t test). (F) Knockdown of rev-erbα abolishes the inhibition of il6 mRNA abundance seen with GSK4112 (n = 3, t test).
Are Circadian Rhythms the Code of Hypothalamic-Immune Communication? Insights from Natural Killer Cells

Alvaro Arjona · Dipak K. Sarkar

Table 1  Effects of circadian gene disruption on the immune system

<table>
<thead>
<tr>
<th>Gene</th>
<th>Manipulation</th>
<th>Effect</th>
</tr>
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<tbody>
<tr>
<td>Per2</td>
<td>Mutation</td>
<td>Loss of daily rhythm of IFN-γ [111]</td>
</tr>
<tr>
<td>Per2</td>
<td>Mutation</td>
<td>Resistance to LPS-induced endotoxic shock [112]</td>
</tr>
<tr>
<td>Per2</td>
<td>Knockdown by RNAi</td>
<td>Decrease in granzyme B and perforin levels [74]</td>
</tr>
<tr>
<td>Bmal1</td>
<td>Deletion</td>
<td>Impaired B cell development [113]</td>
</tr>
<tr>
<td>Clock</td>
<td>Mutation</td>
<td>Suppression of daily rhythms in circulating leukocytes [114]</td>
</tr>
<tr>
<td>Clock</td>
<td>Mutation</td>
<td>Reduced expression levels of multiple immune-related genes [115]</td>
</tr>
</tbody>
</table>
Clock Gene Expression during Chronic Inflammation Induced by Infection with *Trypanosoma brucei brucei* in Rats

Gabriella B. S. Lundkvist†, 1, Michael T. Sellix†, Mikael Nygård‡, Erin Davis†, Marty Straume§, Krister Kristensson†, and Gene D. Block†

†Swedish Medical Nanoscience Center, Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden
‡Department of Biology, University of Virginia, Charlottesville, VA

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**Circadian Rhythms of Clock Gene Expression in SCN Are Not Affected by Trypanosome Infection, but Clock Gene Expressions in Pituitary Gland, Pineal Gland, and Spleen Are Differentially Altered**
Focused Review

Neuroimmunology of the circadian clock

Andrew N. Coogan*, Cathy A. Wyse

Table 1 – Expression of cytokines, and their receptors, in the master SCN pacemaker and central, semi-autonomous oscillators of the circadian system of the rodent brain

<table>
<thead>
<tr>
<th>Cytokine/receptor</th>
<th>Location</th>
<th>Method of detection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>SCN</td>
<td>Immunohistochemistry</td>
<td>Lechan et al. (1990)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>SCN</td>
<td>Immunohistochemistry</td>
<td>Breder et al. (1993)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>SCN</td>
<td>RT-PCR</td>
<td>Sadki et al. (2007)</td>
</tr>
<tr>
<td>IL-6</td>
<td>SCN</td>
<td>In situ hybridisation</td>
<td>Gonzalez-Hernandez et al. (2006)</td>
</tr>
<tr>
<td>TGF-β1 receptor</td>
<td>SCN</td>
<td>In situ hybridisation</td>
<td>Prevot et al. (2000)</td>
</tr>
<tr>
<td>IFN-γ receptor</td>
<td>SCN</td>
<td>Immunohistochemistry</td>
<td>Lunqvist et al. (1998, 1999)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>SCN</td>
<td>RT-PCR</td>
<td>Sadki et al. (2007)</td>
</tr>
<tr>
<td>CLC</td>
<td>SCN</td>
<td>In situ hybridisation</td>
<td>Kraves and Weitz (2006)</td>
</tr>
<tr>
<td>IL-2/15</td>
<td>Habenula</td>
<td>In situ hybridisation</td>
<td>Petitto and Huang (2001)</td>
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<tr>
<td>IL-18</td>
<td>Habenula</td>
<td>In situ hybridisation</td>
<td>Sugama et al. (2002)</td>
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<td>IL-6/IL-6 receptor</td>
<td>Habenula</td>
<td>In situ hybridisation</td>
<td>Schobitz et al. (1992)</td>
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<td>Dorsomedial hypothalamus</td>
<td>In situ hybridisation</td>
<td>Schobitz et al. (1992)</td>
</tr>
<tr>
<td>IL-1 type 1 receptor</td>
<td>Dorsomedial hypothalamus</td>
<td>Immunohistochemistry</td>
<td>Hassanain et al. (2005)</td>
</tr>
<tr>
<td>IL-2 receptor</td>
<td>Dorsomedial hypothalamus</td>
<td>Immunohistochemistry</td>
<td>Bhatt et al. (2005)</td>
</tr>
<tr>
<td>IL-2/IL-2 receptor</td>
<td>Arcuate nucleus</td>
<td>In situ hybridisation</td>
<td>Lapchak (1992)</td>
</tr>
<tr>
<td>IL-1 type 1 receptor</td>
<td>Arcuate nucleus</td>
<td>In situ hybridisation</td>
<td>Ericsson et al. (1995)</td>
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<tr>
<td>IL-2</td>
<td>Arcuate nucleus</td>
<td>PCR</td>
<td>Tanebe et al. (2000)</td>
</tr>
<tr>
<td>TGF-β receptor</td>
<td>Arcuate nucleus</td>
<td>In situ hybridisation</td>
<td>Prevot et al. (2000)</td>
</tr>
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<td>IL-1β</td>
<td>Olfactory bulb</td>
<td>In situ hybridisation</td>
<td>Sandtow et al. (1990)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Olfactory bulb</td>
<td>In situ hybridisation</td>
<td>Lim and Brunjes (1999)</td>
</tr>
<tr>
<td>IL-2/IL-2 receptor</td>
<td>Olfactory bulb</td>
<td>RT-PCR/immunohistochemistry</td>
<td>Wang et al. (2001)</td>
</tr>
</tbody>
</table>
Focused Review

Neuroimmunology of the circadian clock

Andrew N. Coogan*, Cathy A. Wyse

Fig. 1 – Schematic illustrating some putative pathways by which circadian output from the SCN may be influenced, and in turn influence, levels of peripheral and central cytokines. SCN output via the autonomic system, hypothalamic-pituitary axis activation or melatonin secretion (all involving SCN projections to the paraventricular nucleus (PVN) of the hypothalamus) impacts on cytokine production by peripheral immune cells, some of which have also been shown to possess an endogenous clock. Circulating cytokines may then feedback to the SCN via induction of prostaglandins (PGEs) in the cerebral vasculature, activation at the circumventricular organs or via brainstem pathways (involving the nucleus of the solitary tract (NTS)) following vagal afferent activation.
Sleep, Immunity, and Circadian Clocks: A Mechanistic Model

Thomas Bollinger\textsuperscript{a} Annalena Bollinger\textsuperscript{b} Henrik Oster\textsuperscript{c} Werner Solbach\textsuperscript{a}

\textsuperscript{a}Institute of Medical Microbiology and Hygiene, University of Luebeck, Luebeck. \textsuperscript{b}Institute for Immunobiology, Research Center Borstel, Borstel, and \textsuperscript{c}Circadian Rhythms Group, Max Planck Institute of Biophysical Chemistry, Goettingen, Germany

**Fig. 1.** Influence of sleep on rhythmic hormonal and immune parameters. Peripheral blood was drawn from 7 healthy young men who either slept normally (solid line) or were sleep deprived for 1 day (dashed line). Average circadian serum profiles of

**Fig. 3.** Clock-sleep-immune model. The SCN is a key regulator of
Crosstalk between the circadian clock circuitry and the immune system

Humans (diurnal)

Rodents (nocturnal)

Sleep, Jet lag, Shift work, Aging...

Circadian disruption

MASTER CLOCK

SCN

Pro-inflammatory cytokines

Macrophages, Monocytes

Mast cells

Nucleus

CLOCK, BMAL

PER, CRY

Cytosol

Th1 responses (Rheumatoid arthritis)

Th2 responses (Allergy)

CELL CLOCKS

Clock controlled genes, STAT3, STAT5, NFκB
Rhythms in cellular functions

B cells, CD4 T cells (blood numbers)

SNS

Epinephrine, Norepinephrine

NK cells (blood numbers, cell functions in blood & spleen)
Cytotoxic defense

Low grade systemic inflammation,
Compromised adaptive immunity?
Increased autoimmunity?
Increased allergic responses?
Decreased tumor surveillance?

Synchronizing signals, in addition to effects of behavioral cycles (sleep-wake, rest-activity, fasting-feeding)

HPA axis

Cortisol

Sleep loss

LPS responsivity

IL-1

TNFα

GH, Prolactin, (Melatonin)

REST PERIOD

ACTIVE PERIOD

Nicolao Cermakian, Tanja Lange, Diego Golombek, Dipak Sarkar, Atsuhiro Nakao, Shigenobu Shibata, Gianluigi Mazzoccoli
Dziękuję za uwagę