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A TIME TO KILL, AND A TIME TO HEAL. PATHOPHYSIOLOGICAL INTERACTIONS BETWEEN THE CIRCADIAN AND THE IMMUNE SYSTEMS.

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Daily variations in health.

Over 2400 years ago, Hippocrates taught his associates that regularity was a sign of health, and that irregular body functions or habits indicated or promoted an unhealthy condition. A few centuries later, Soranus from Efeso (II century AC) reported that asthma attacks are observed frequently at night and during winter (Reinberg 2006) . These and other early hypotheses and facts suggested that immune-related processes undergo a temporal regulation that is related with the appearance of disease. In 1960 (around the time when chronobiology was beginning to be considered a scientific discipline), experiments with rodents conducted by Franz Halberg demonstrated a circadian variation in susceptibility to lethal doses of bacterial lipopolysaccharide (LPS) endotoxin, with higher mortality occurring during the day (Halberg *et al.* 1960) (**Figure 1A**). Although this experiment is one of the foundations for chronopharmacology and chronotoxicology, it was not repeated or extended until quite recently (Liu *et al.* 2006 , Marpegan *et al.* unpublished). Indeed, this daily pattern in lethality was observed in a more recent experiment using tumor necrosis factor alpha (TNF-α), one of the main LPS-induced pro-inflammatory cytokines (Hrushesky *et al.* 1994) (**Figure 1A**). While Halberg (1960) provided

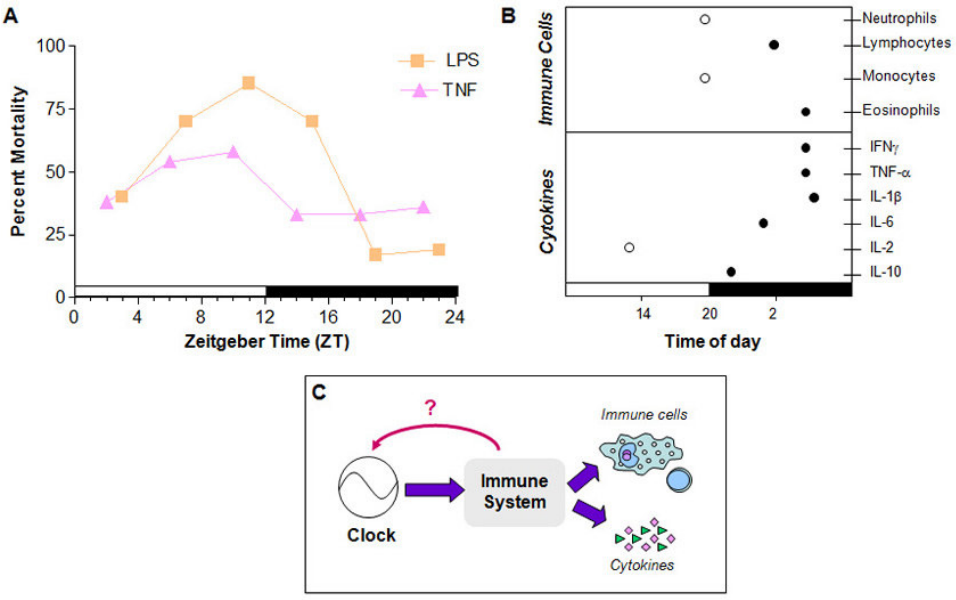

Figure 1*. Daily variations in the immune system. (A)** *24-h changes in the susceptibility to both LPS (modified from Halberg, 1960) and TNF-α (modified from Hrushevsky, 1994) injections delivered at different hours of the day (zeitgeber time 12 is defined as the time of lights off). Higher diurnal lethality depicts a strong circadian modulation of LPS-induced sepsis, which is also observed using TNF-α stimulation, an endogenous pro-inflammatory factor.* ***(B) *Diurnal and nocturnal distribution of peak times (acrophase map) of immune parameters, number of plasmatic immune cells and cytokine levels in humans (modified from Haus and Smolensky, 1999).* ***(C)*** *Model for the circadian regulation of immune system and the putative feedback of immune factors on clock activity.*

the first experimental evidence about daily variations in immune response, current clinical data show a strong correlation between time of day and illness manifestation or immune activity. For example, symptoms of rheumatoid arthritis occur during the morning (Katz *et al.* 2002) most asthma attacks during the night (Reinberg 2006) , and the effects of immunization also change with daytime (Langlois *et al.* 1995) . Taken together, these reports suggest a strong regulation exerted by the circadian clock on the immune system, which will be reviewed in this article. Moreover, clock-controlled rhythms in several variables exert a feedback regulation on the circadian oscillator itself, a mechanism that we shall also consider in this paper.

Daily rhythms in the immune system.

Biological clocks drive 24-h (circadian) rhythms in most physiological and behavioral variables, including body temperature, sleep, hormonal secretion and locomotor activity. In mammals, the hypothalamic suprachiasmatic nuclei (SCN) are considered to be the master circadian pacemaker, which is entrained by environmental variables such as the light-dark cycle and is therefore synchronized with the solar day. A hallmark of the SCN pacemaker is its ability to orchestrate physiological rhythms maintaining stable phase-relationships, by neural efferents towards both neuroendocrine and autonomic neurons of the paraventricular nuclei (PVN) and the sub-PVN region in the hypothalamus (Buijs *et al.* 2003) . Although the particular efferent pathways are not completely understood, daily cycles in immune-related variables determine the rhythmic variation in the intensity of inflammatory processes, whether in response to environmental stimuli or in the course of autoimmune diseases. A variety of immune parameters manifest daily fluctuations, as for instance the levels of white blood cells in peripheral blood. In humans, the number of lymphocytes, eosinophils and basophile granulocytes peaks during the night, whereas monocytes and neutrophile levels fall during the day (reviewed in Haus and Smolensky 1999) (**Figure 1B**). In addition, major humoral immune responses undergo circadian changes, as antibodies titer after immunization, or peak concentration of three major classes of immunoglobulins (Haus and Smolensky 1999) Rhythms in plasmatic levels of pro-inflammatory cytokines, as well peptide hormones produced and secreted by immune cells, were also reported. Interleukin-1β (IL-1β), 6, 10 and 12, TNF-α, and interferon gamma (IFN-γ) exhibit in most cases high levels during the night and early morning in the human (**Figure 1B**) (Petrovsky and Harrison 1997) . The rise in cytokine plasmatic levels may at least in part explain the exacerbation of immuno-inflammatory disorders at these daytimes. Furthermore, the fact that nocturnal rodents experienced rhythms with opposite phase to diurnal ones suggests a relationship between behavioral state and immune activity. Although there are some contradictory data, it can be generalized that the levels of most inflammatory immune cells and factors are enhanced during the rest phase (i.e., night for humans, day for nocturnal rodents).

Autonomic and neuroendocrine circadian control of the immune system.

Despite the great amount of evidence describing immune rhythms, to date there are no consistent experimental data suggesting a direct link between the SCN clock and such rhythms. The principal outputs of the circadian clock for physiological daily regulation consist of their efferencies to hypothalamic centers involved in autonomic and

neuroendocrine control. Neurons projecting from these centers towards sympathetic and parasympathetic systems send circadian information to different organs, including pineal gland, adrenals, liver, etc. Primary (bone marrow and thymus) and secondary (spleen and lymph nodes) lymphoid organs are richly supplied with autonomic (mainly sympathetic) and sensory innervation (Mignini *et al.* 2003) . The peak number of immune cells may be temporally regulated by the autonomic nervous system, since cells that are abundant during the day are highly concentrated in adrenergic (rather than cholinergic) receptors, and the inverse occur in cells which peak at night (Suzuki *et al.* 1997) . Importantly, there is direct circadian control on hypothalamic neurons that release corticotrophin-releasing hormone (CRF). CRF activates the pituitary-adrenal axis via adrenocorticotrophin hormone (ACTH), producing a morning peak in plasmatic corticosteroids level to enhance stress responses in the beginning of activity. Corticosteroids and catecholamines are strong modulators of the immune system, reducing pro-inflammatory activity; moreover, the corticosteroid peak is usually taken to explain rhythms in immune parameters. In addition, the immune system feedbacks on autonomic abdominal vagus nerves by the paracrine action of cytokines, which also interact with receptors in vagal sensory neurons (Konsman *et al.* 2002) . Changes in immune function can also influence the expression of neural receptors in lymphoid organs (Mignini *et al.* 2003) . Circulating cytokines can exert endocrine effects on certain hypothalamic centers and on endocrine cells, which in turn regulate cytokine production. The production and release of CRF, ACTH and corticosteroids is also stimulated in response to pro-inflammatory cytokines (especially IL-1) secreted at the site of injury, or in the course of immune challenges, to promote anti-inflammatory responses in a feedback regulatory mechanism (Haus and Smolensky 1999) . Thus, the immune system acts through the production of cytokines which signals the exposure to antigen to the central nervous and endocrine systems. On the other hand, while cortisol acts as immune suppressor, the pineal hormone melatonin might enhance inflammation by increasing IFN-γ and IL-1 production, and antagonizes the immunosuppressive effects of cortisol (Haus and Smolensky 1999) . Melatonin is under strong circadian control through the autonomous nervous system, and therefore it can be partially responsible for immune rhythms. Also, since plasmatic levels of this hormone encode daylenght to drive seasonal reproduction and growth, it is not surprising to find circannual changes in immune responses. In addition, factors that affect neuroendocrine rhythms (i.e. physical exercise, sleep deprivation, etc.), can also modulate immune functions. These interactions produce a complex framework of circadian-immune cross-talking, also taking into account an additional neuroendocrine modulation.

The immune system and sleep.

It is a common perception that we are more susceptible to infections when we are deprived of sleep and that many infections seem to cause increased somnolence. It could be argued that sleep somehow regulates the immune functions but, in addition, the immune system could also be able to modify the sleep-wake cycle.

"Sleep → Immune" Interaction.

Extensive data support the idea that immunological variables are influenced by the sleep-wake cycle. Natural killer (NK) cell activity, antigen uptake, circulating immune complexes, secondary antibody responses are altered by sleep loss (Krueger *et al.* 2003)

Also, IL-1β and TNF-α levels increase during sleep deprivation, although other cytokines exhibit no sleep-related variations (Majde and Krueger 2005). These immune modifications induced by sleep deprivation seem to be unrelated with the endocrine system, or at least with cortisol and ACTH, because the circadian rhythms of these hormones remain unaltered.

Sleep-deprived humans immunized with the influenza A virus have lower levels of virus-specific titres than non-deprived individuals. In rats, prolonged sleep deprivation leads to an immune impairment that can cause death. In humans, the effects of sleep loss on infections and mortality are positively correlated with aging (Bryant *et al.* 2004) .

In addition, sleep deprivation affects the rhythms of immune cells subsets, which are under the control of both the sleep cycle and the circadian pacemaker (Born *et al.* 1997) .

"Immune → Sleep" Interaction.

There are many evidences supporting the observation that sleep is increased during infection. Several human illnesses specifically alter the sleep-wake cycle. Trypanosomiasis (sleeping sickness) is an unique model of sleep and infection where sleep recording do not show increased overall duration but have substancial disturbances in the circadian regulation of sleep-wake cycle. Animal models of Chagas disease (caused by the parasite *Trypanosoma cruz*i) also exhibit significant changes in their circadian rest-activity rhythms (Fernandez Alfonso *et al.* 2003) . Allergic patients have higher serum levels of IL-1β, IL-4 and IL-10, which correlate with faster sleep onset, as well as an increased latency to Rapid Eye Movement (REM) sleep onset with a shorter duration (Bryant *et al*. 2004) .

Experiments with animals demonstrate an increase in the duration of Slow Wave Sleep (SWS) following infection. Administration of bacterial components (muramyl peptides and LPS) has somnogenic effects (dose-dependent increases in SWS duration and decreased REM sleep) which can be separated from pyrogenic response (low doses induce somnogenic effects but not fever). Immune sleep modulations are related with the age, and this may be related with immune senescence.

IL-1β and TNF-α appear to be the most important immune factors in sleep regulation. Their administration induces an increase in the amount of time spent in Non-Rapid Eye Movement Sleep (NREMS) in different species. Moreover, antibodies to IL-1 and TNF inhibit the spontaneous NREMS. Mutant mice lacking IL-1 or TNF receptor sleep less than littter-mate controls. In summary, most pro-inflammatory cytokines seem to be somnogenic, whereas most anti-inflammatory cytokines are not (Krueger *et al*. 2003, Majde and Krueger 2005) ,

Both IL-1 and TNF activate Nuclear Factor kappa B (NF-κB), which in turn induces the release of these cytokines (closing a positive feedback loop). Inhibitors of this transcription factor tend to inhibit sleep, while sleep deprivation induces NF-κB in several brain areas. Taken together, these data support the fact that NF-κB is also a key factor in sleep regulation (Bryant *et al*. 2004) .

All of these evidences indicate that the immune system is able to modify sleep in pathological and physiological conditions. Cytokines effects on sleep appear to be

mediated by other humoral factors in the brain, such as GHRH that is induced by pro-inflamatory cytokines and also increases NREMS.

Immune-circadian interactions.

There is a substantial amount of information regarding the circadian modulation of many immunological variables. Furthermore, the sleep–wake cycle (one of the most evident circadian rhythms) is modified by pro-inflammatory factors (Krueger *et al.* 2001) . However, there are almost no data about the possible effect of immune system on the circadian clock itself. Our aim in the laboratory is to investigate how these factors modify critical clock functions, such as phase resetting mechanisms.

Immune effects on the clock

There are a few *in vivo* experiments that study this interaction, which use high doses of LPS or pathological states (sepsis or infection with blood-borne parasites) that severely affect circadian output (Bauhofer *et al.* 2001, Bauhofer *et al.* 2002, Bentivoglio *et al.* 1994, Ebong *et al.* 1999, Halberg *et al*. 1960) . Some *in vitro* results show that pro-inflammatory factors modify clock-genes expression (Motzkus *et al.* 2002, Nava *et al.* 2000) .

We chose to use intraperitoneal (i.p.) administration of low, subpyrogenic doses of lipopolysaccharide (LPS) as an immune stimulus to evaluate the physiological effects of immune factors on clock-controlled locomotor activity rhythms. The major effect we observed was a change in the phase of the rhythm. When we delivered LPS i.p. to mice running on wheels in constant dark conditions (DD) at different circadian times (CT, with CT 12 defined as the time of locomotor activity onset), we discovered that it induces phase delays only when was injected at CT15 (without any effects on body temperature) (**Figure 2**). This was the first *in vivo* evidence of the response of the circadian system to an immune challenge such as LPS and, since we used relatively low doses of endotoxin, suggests the existence of a feedback pathway from immune effectors Into the circadian

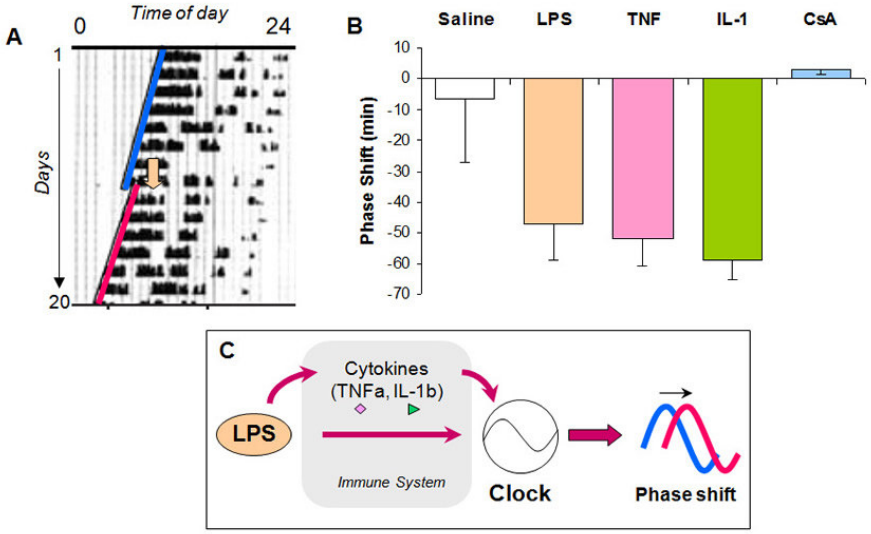

Figure 2. Effect of LPS and cytokines on circadian phase. (A) *Actogram representing the running-wheel activity pattern; each row shows the amount of activity distributed along 24 hours, and plotted in a vertical succession of days. Mice were kept in constant dark conditions (DD), with circadian time (CT) 12 defined as the time for activity onset (used as reference phase). Mice were treated with a subpyrogenic dose of LPS at CT 15 (orange arrow), which induced a phase delay in the onset of locomotor activity.* ***(B)*** *Phase shifts in response to saline, LPS (ip), TNFα (icv), IL-1β (icv) and ciclosporin (CsA, i.p.) at CT 15 (3 hours after activity onset). Treatments with pro-inflamatory factors (LPS and cytokines) induce similar phase delays in the activity rhythm of mice under constant dark conditions.* ***(C)*** *Model for the immune regulation of the circadian clock. LPS may be inducing phase shifts either directly or by using cytokines as intermediary signaling molecules.*

system (Marpegan *et al.* 2005). The most likely candidates to mediate the central circadian response to bacterial endotoxin are the proinflamatory cytokines IL-1β and TNF-α, so we administered these cytokines intracerebroventricularly (i.c.v.) and we found that they also induce phase delays in locomotor activity at CT15 in mice (**Figure 2A, B**) (Marpegan *et al.* 2005 and unpublished data). On the other hand, immune suppression with Ciclosporin (CsA) has no effect on clock phase at CT15 (but there are CsA-induced phase changes at other times of the day, suggesting an opposite effect of immune activating or inhibiting factors) (Golombek *et al.* 1998) . Therefore, all these evidences suggest that LPS may act on the clock by inducing the release of cytokines which finally act on the SCN (**Figure 2C**).

A classical immune transcription factor, NF-kappa B, in the immune-circadian interaction.

Following our initial description of the immune modulation of the central circadian clock, we wanted to know whether NF-κB, an immune-related transcription factor, was involved in this modification. Indeed, we found that NF-κB inhibition blocks both LPS- and light-induced phase shifts of circadian rhythms (**Figure 3A, B**). In addition, LPS- and light-

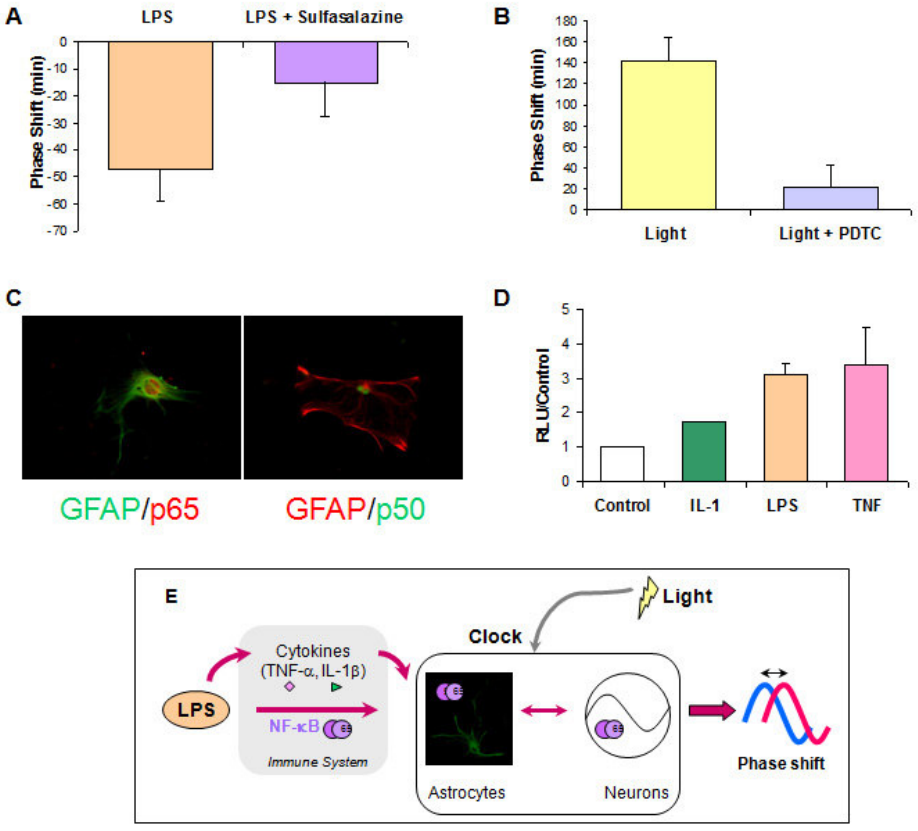

Figure 3*. Involvement of NF-kappa B in circadian synchronization.** ***(A) *Administration of a specific NF-kB-inhibitor (sulfasalazine) blocks the phase delays induced by LPS at CT 15 in mice.* ***(B)*** *Injection of PDTC (pyrrolidine-dithiocharbamate), another NF-kB inhibitor, blocks light-induced phase advances in hamsters. The fact that NF-kappa B blockers have effects on LPS- and light- induced phase shifts indicates that this transcription factor is a common step in circadian clock entrainment.* ***(C)*** *Double immunofluorescence micrography of Glial Fibrillary Acidic Protein (GFAP, astroglia marker) and NF-kB subunits (p65 or p50) in astroglial SCN cell culture.* ***(D)*** *NF-kB activity, measured as bioluminescence induced by a kB promotor-luciferase plasmid, was increased by LPS, TNF or IL-1.* ***(E)*** *Model of the immune regulation of the circadian clock. LPS-induced phase shifts are mediated by NF-kB but could be elicited in the periphery or in the SCN itself. Since light- and LPS-induced phase shifts are not additive (data not shown) might indicate a common NF-kappa B-related pathway in the SCN. Moreover, NF-kB activity in SCN astroglial cells is increased in response to immflamatory factors.*

induced phase shifts are not additive (data not shown) suggesting there is some interaction between both pathways. This interaction could be at the NF-κB level in the SCN. In order to test this possibility, we analyze the presence of this factor at the SCN and found NF-κB expression and activity in SCN homogenates (Marpegan *et al.* 2004) . The next step was to evaluate the cellular substrate of this circadian-immune interaction, under the hypothesis that astroglia was particularly relevant for this mechanism. The SCN has a dense and cyclic immunorreactivity for Glial Fibrillary Acidic Protein (GFAP, specific marker of astroglial cells), and astrocytes have been shown to be mediators of immune mechanisms in several experimental models (Aschner 1998, Dong and Benveniste 2001). We have found NF-κB expression in astrocytes *in vitro* (**Figure 3C**) and in brain slices (data not shown); in addition, NF-κB was activated in response to LPS and pro-inflammatory cytokines in SCN astroglial cultures (Leone *et al.* 2006) (**Figure 3D**). **Figure 3E** shows our model of immune regulation of the clock. A framework for the circadian-immune interactions is shown in **Figure 4**, including the paths used by these systems.

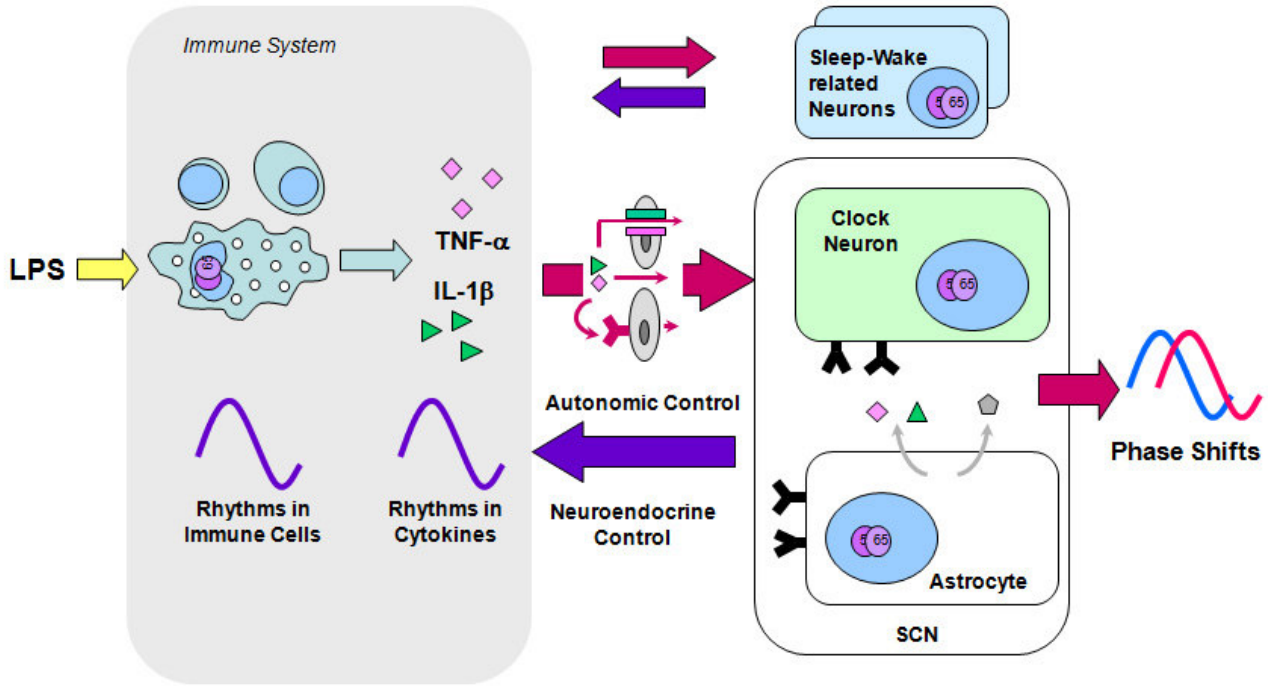

***Figure 4*. Circadian-immune interaction.** *When the immune system is challenged (by microorganisms or their components) there is a release of specific cytokines that elicit the immune response (e.g., TNF-α and IL-1β). These cytokines (and also LPS) may affect the sleep-wake cycle and/or the circadian clock using differents ways. (1) They can enter through sites which lack the blood-brain barrier (BBB), such as the*

organum vasculosum of lamina terminalis and the median eminence of the hypothalamus. (2) They can enter the brain parenchyma through specific transporters in the BBB. (3) BBB endothelial cells express cytokines receptors and are able to produce cytokines (and other mediators) in the brain. (4) They can signal the brain through the vagus nerve. (5) Cytokines can use endocrine signals to affect the brain. Cerebral cytokines may affect SCN astroglial cells, which might release other cytokines or factors that stimulate clock neurons, in all cases through activation of a NF-κB-related mechanism.

The circadian control of the immune system (blue arrow) can be mediated by the neuroendocrine system or via the autonomic nervous system, inducing rhythms in cytokines levels and immune cell number and activity. Modulation of the immune system by sleep can be mediated by the same mechanisms.

Concluding remarks.

Circadian rhythms are ubiquitous in nature, and control temporal order in most (if not all) physiological systems. Indeed, most immune factors and processes are under diurnal control, orchestrated by the circadian system, although the efferent pathways that control these cycles are not completely understood.

In addition, circadian disorders are usually associated with disease, and temporal disarrangements in immune parameters are closely related to the onset or development of pathological mechanisms. In this paper we argue that the circadian-immune interaction can operate in two directions. First, daily cycles in immune parameters are controlled by the central circadian pacemaker located at the hypothalamic SCN. On the other hand, these same temporal variations might be useful for a fine-tuning of the system by affecting receptors located in the circadian clock. We have provided evidence suggesting that this latter interaction might be mediated by glial cells that respond to peripheral of central cytokines which by different transcriptional activators affect molecular circadian rhythms. Indeed, the precise knowledge of this circadian-immune interaction might be extremely useful for the understanding of why many infections and illnesses affect circadian rhythms (including the sleep-wake cycle) and greatly decrease the patients' quality of life.

Besides a better understanding of the physiological regulation of circadian rhythmicity, the chronobiological basis of the times to kill and the times to heal will therefore provide a novel basis for diagnosis and therapeutical approaches in order to treat disease.

REFERENCES

1. **Aschner M**. Immune and inflammatory responses in the CNS: modulation by astrocytes. *Toxicol Lett* 102-103: 283-287, 1998.

2. **Bauhofer A, Witte K, Celik I, Pummer S, Lemmer B, and Lorenz W**. Sickness behaviour, an animal equivalent to human quality of life, is improved in septic rats by G-CSF and antibiotic prophylaxis. *Langenbecks Arch Surg* 386: 132-140, 2001.

3. **Bauhofer A, Witte K, Lemmer B, Middeke M, Lorenz W, and Celik I**. Quality of life in animals as a new outcome for surgical research: G-CSF as a quality of life improving factor. *Eur Surg Res* 34: 22-29, 2002.

4. **Bentivoglio M, Grassi-Zucconi G, Peng ZC, and Kristensson K**. Sleep and timekeeping changes, and dysregulation of the biological clock in experimental trypanosomiasis. *Bull Soc Pathol Exot* 87: 372-375, 1994.

5. **Born J, Lange T, Hansen K, Molle M, and Fehm HL**. Effects of sleep and circadian rhythm on human circulating immune cells. *J Immunol* 158: 4454-4464, 1997.

6. **Bryant PA, Trinder J, and Curtis N**. Sick and tired: Does sleep have a vital role in the immune system? *Nat Rev Immunol* 4: 457-467, 2004.

7. **Buijs RM, van Eden CG, Goncharuk VD, and Kalsbeek A**. The biological clock tunes the organs of the body: timing by hormones and the autonomic nervous system. *J Endocrinol* 177: 17-26, 2003.

8. **Dong Y, and Benveniste EN**. Immune function of astrocytes. *Glia* 36: 180-190, 2001.

9. **Ebong S, Call D, Nemzek J, Bolgos G, Newcomb D, and Remick D**. Immunopathologic alterations in murine models of sepsis of increasing severity. *Infect Immun* 67: 6603-6610, 1999.

10. **Fernandez Alfonso T, Celentano AM, Gonzalez Cappa SM, and Golombek DA**. The circadian system of Trypanosoma cruzi-infected mice. *Chronobiol Int* 20: 49-64, 2003.

11. **Golombek DA, Katz M, and Ralph MR**, Calcineurin modulates circadian rhythms and circadian responses to light, in *Biological Clocks. Mechanisms and Applications*, edited by Touitou, Y, pp. 221-224, Elsevier Science B.V., Paris, 1998.

12. **Halberg F, Johnson EA, Brown BW, and Bittner JJ**. Susceptibility rhythm to E. coli endotoxin and bioassay. *Proc Soc Exp Biol Med* 103: 142-144, 1960.

13. **Haus E, and Smolensky MH**. Biologic rhythms in the immune system. *Chronobiol Int* 16: 581-622, 1999.

14. **Hrushesky WJ, Langevin T, Kim YJ, and Wood PA**. Circadian dynamics of tumor necrosis factor alpha (cachectin) lethality. *J Exp Med* 180: 1059-1065, 1994.

15. **Katz M, Marpegan L, and Bekinschtein T**, Ritmos diarios y circadianos en el sistema inmunológico, in *Cronobiología Humana*, edited by Golombek, DA, pp. 119-129, Universidad Nacional de Quilmes Ediciones, Bernal, 2002.

16. **Konsman JP, Parnet P, and Dantzer R**. Cytokine-induced sickness behaviour: mechanisms and implications. *Trends Neurosci* 25: 154-159, 2002.

17. **Krueger JM, Majde JA, and Obal F**. Sleep in host defense. *Brain Behav Immun* 17 Suppl 1: S41-47, 2003.

18. **Krueger JM, Obal FJ, Fang J, Kubota T, and Taishi P**. The role of cytokines in physiological sleep regulation. *Ann N Y Acad Sci* 933: 211-221, 2001.

19. **Langlois PH, Smolensky MH, Glezen WP, and Keitel WA**. Diurnal variation in responses to influenza vaccine. *Chronobiol Int* 12: 28-36, 1995.

20. **Leone MJ, Marpegan L, Bekinschtein TA, Costas MA, and Golombek DA**. Suprachiasmatic astrocytes as an interface for immune-circadian signalling. *J Neurosci Res* 84: 1521-1527, 2006.

21. **Liu J, Mankani G, Shi X, Meyer M, Cunningham-Runddles S, Ma X, and Sun ZS**. The circadian clock Period 2 gene regulates gamma interferon production of NK cells in host response to lipopolysaccharide-induced endotoxic shock. *Infect Immun* 74: 4750-4756, 2006.

22. **Majde JA, and Krueger JM**. Links between the innate immune system and sleep. *J Allergy Clin Immunol* 116: 1188-1198, 2005.

23. **Marpegan L, Bekinschtein TA, Costas MA, and Golombek DA**. Circadian responses to endotoxin treatment in mice. *J Neuroimmunol* 160: 102-109, 2005.

24. **Marpegan L, Bekinschtein TA, Freudenthal R, Rubio MF, Ferreyra GA, Romano A, and Golombek DA**. Participation of transcription factors from the Rel/NF-kappa B family in the circadian system in hamsters. *Neurosci Lett* 358: 9-12, 2004.

25. **Mignini F, Streccioni V, and Amenta F**. Autonomic innervation of immune organs and neuroimmune modulation. *Auton Autacoid Pharmacol* 23: 1-25, 2003.

26. **Motzkus D, Albrecht U, and Maronde E**. The human PER1 gene is inducible by interleukin-6. *J Mol Neurosci* 18: 105-109, 2002.

27. **Nava F, Carta G, and Haynes LW**. Lipopolysaccharide increases arginine-vasopressin release from rat suprachiasmatic nucleus slice cultures. *Neurosci Lett* 288: 228-230, 2000.

28. **Petrovsky N, and Harrison LC**. Diurnal rhythmicity of human cytokine production: a dynamic disequilibrium in T helper cell type 1/T helper cell type 2 balance? *J Immunol* 158: 5163-5168, 1997.

29. **Reinberg A**, Ritmos biológicos en el sistema respiratorio. Asma Nocturna., in *Cronobiología Básica y Clínica.*, edited by Madrid, JA, and Rol de Lama, A, pp. 631-655, Editec@red, Madrid, 2006.

30. **Suzuki S, Toyabe S, Moroda T, Tada T, Tsukahara A, Iiai T, Minagawa M, Maruyama S, Hatakeyama K, Endoh K, and Abo T**. Circadian rhythm of leucocytes and lymphocytes subsets and its possible correlation with the function of the autonomic nervous system. *Clin Exp Immunol* 110: 500-508, 1997.