MINI REVIEW
Melatonin and viral infections

Abstract: The therapeutic effects of melatonin against viral infections, with emphasis on the Venezuelan equine encephalomyelitis (VEE), are reviewed. Melatonin has been shown to prevent paralysis and death in mice infected with the encephalomyocarditis virus and to decrease viremia. Melatonin also postpones the onset of the disease produced by Semliki Forest virus inoculation and reduces the mortality of West Nile virus-infected mice stressed by either isolation or dexamethasone injection. An increase in the host resistance to the virus via a peripheral immunostimulatory activity is considered responsible for these effects. It has also been demonstrated that melatonin protects some strains of mink against Aleutian disease, and prevents the reduction of B- and T-cells as well as Th1 cytokine secretion in mice infected with leukemia retrovirus. In VEE-infected mice, melatonin postpones the onset of the disease and death for several days and reduces the mortality rate. This protective effect seems to be due to the increase in the production of interleukin-1β (IL-1β), as 100% of the infected mice treated with melatonin die when IL-1β is blocked with antimurine IL-1β antibodies. Although melatonin administration raises serum levels of tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ), the mortality observed in neutralization experiments with the corresponding anticytokine antibodies, suggests that neither TNF-α nor IFN-γ are essential for the protective effect of melatonin on murine VEE virus infection. Melatonin treatment also enhances the efficiency of immunization against the VEE virus. Reactive oxygen species have been implicated in the dissemination of this virus, and their deleterious effects may be diminished by melatonin. This indole inhibits nitric oxide synthetase activity and it is a potent scavenger of nitric oxide, which also plays an important role in the spread of the VEE virus. In conclusion, the immunomodulatory, antioxidant, and neuroprotective effects of melatonin suggest that this indole must be considered as an additional therapeutic alternative to fight viral diseases.

Ernesto Bonilla[1,2], Nereida Valero[1], Leonor Chacín-Bonilla[1] and Shirley Medina-Leendertz[2]

[1]Instituto de Investigaciones Clínicas 'Dr. Américo Negrette', Facultad de Medicina, Universidad del Zulia, Maracaibo; [2]Instituto de Investigaciones Biomédicas, FUNDACITE-Zulia, Maracaibo, Venezuela

Address reprint requests to Ernesto Bonilla, Instituto de Investigaciones Clínicas 'Dr. Américo Negrette', Apartado Postal 1151, Maracaibo, Venezuela.
E-mail: ebonillaro@yahoo.com

Introduction

The pineal gland is a neurochemical transducer capable of perceiving basic environmental information and integrating it with the synthesis and release of a variety of agents of which melatonin (*N*-acetyl-5-methoxytryptamine) is the most thoroughly studied. This secretory product appears to be involved in synchronizing circadian and seasonal timing of several physiological and behavioral processes [1–4]. The melatonin rhythm is certainly an important efferent message from the suprachiasmatic nuclei signal for influencing the organism's circadian system [5].

Melatonin is also a powerful and effective hydroxyl radical scavenger, which provides on-site protection against oxidative damage to cell components [6]. It also scavenges the peroxyl radical generated during lipid peroxidation with an activity that is reportedly greater than that of vitamin E [7]. Additionally, in vivo it stimulates several antioxidative enzymes including glutathione peroxidase, thereby potentiating its antioxidant properties [6]. Thus, melatonin seems to be an essential component of the antioxidant defense system [8].

It has also been shown that melatonin has an important role in the immune function both under physiological and physiopathological conditions [9]. By binding to T-helper cells, the indole gives rise to a cascade of events leading to an increase in immune responsiveness [10, 11]. Several immune functions appear to be modulated by melatonin, including the antitumor defenses of the host [12] and cytotoxicity of natural killer cells [13]. The immune effects induced by melatonin seem to be mediated, at least in part, by endogenous opioid peptides [14]. Exogenous administration of melatonin enhances antibody production [14].

Melatonin binds to membrane receptors [15], which are coupled to two GTP-binding proteins, one sensitive to

pertussis toxin and the other to cholera toxin. Two subtypes of receptors have been described in mammals based on their kinetics and pharmacologic differences of 2-^{125}I-melatonin binding [16] and on their molecular structure [17]. The intimate relationship of melatonin to the immune system is supported by the presence of melatonin receptors in immnunocompetent cells [18]. Specific binding sites for the indoleamine have been described in different immune tissues from many species of birds and mammals [19], including human peripheral blood lymphocytes [20], monocytes [21], and granulocytes [22, 23]. Nuclear receptors for melatonin have also been described in human and murine immunocompetent cells [24–26].

Effect of melatonin on viral infections

The outbreaks of Venezuelan equine encephalomyelitis (VEE) infection in South America in 1995, West Nile encephalitis in the US in 2002, and the severe acute respiratory syndrome (SARS) in 2003, constitute health threats that need to be managed.

Venezuelan equine encephalomyelitis is an important human and equine disease caused by the VEE virus, a mosquito-borne virus of the family Togaviridae and genus *Alphavirus* [27]. Outbreaks occurred in northern South America from the 1920s to the 1970s with thousands of people, horses, and donkeys affected [28–30]. Mice infected with the virus show excitation and hypermotility followed by hypomotility, paralysis, coma, and death [31]. During the 1995 VEE epidemic more than 20 VEE-associated deaths were reported among adults and children in Venezuela and Colombia [32].

West Nile encephalitis is a mosquito-borne viral disease caused by the West Nile virus (WNV), a flavivirus endemic in certain regions of Africa, Asia, and eastern Europe. Patients usually present with headache, fever, gastrointestinal symptoms, maculo-papular rash, and lymphadenopathy. The disease may be fatal, especially in the elderly or in immunodepressed patients [9]. Meningitis and encephalitis may occur leading to death or leaving survivors with neurological deficits [33].

Semliki Forest virus (SFV) is an arbovirus of low pathogenicity in humans [34], but it causes fatal encephalitis in mice [35].

The isolation of a new coronavirus in the respiratory secretions of a patient with SARS and the subsequent demonstration or serological evidence of this virus in other patients with the disease suggest a causal relationship between this virus and SARS [36]. During the initial phase, the symptoms are similar to those of influenza. In all cases, body temperature is 38°C or higher and dry cough is constant, except when there is a superinfection. Dyspnea is frequent, and it occasionally leads to acute respiratory distress with hypoxemia and hypercapnia [37].

Viral infections can produce a generalized immunodepression [38, 39]. Evidence of the ability of melatonin to protect against viral encephalitis was provided by Maestroni et al. [40] who showed that it prevented paralysis and death in mice infected with encephalomyocarditis virus after acute stress. Ben-Nathan et al. [41] reported that administration of melatonin (500 μg/kg from 3 days before

until 10 days after the SFV inoculation) reduced viremia and significantly postponed the onset of the disease by 7–10 days. Melatonin reduced the mortality of SFV (10 plaque-forming units, pfu)-inoculated mice from 100 to 60%. In those inoculated with a higher dose (100 pfu), melatonin reduced mortality by only 20%. In the surviving mice, anti-SFV antibodies were detected 22 days after virus inoculation. WNV infection of mice stressed by either isolation or dexamethasone injection induced mortality of 75 and 50%, respectively, which was reduced by melatonin administration to 31 and 25%, respectively [41].

Melatonin has also been found to protect both a wild-type or demi strain and demi/dark cross strain of mink from Aleutian disease which is produced by a persistent parvoviral infection that results in marked hypergammaglobulinemia and immune complex-mediated lesions of the liver, kidneys, lungs, and arteries [42].

Female C57BL/6 mice infected with the LP-BM5 leukemia retrovirus develop murine acquired immune-deficiency syndrome. This infection inhibits the release of Th1 cytokines, stimulates the secretion of Th2 cytokines, increases hepatic lipid peroxidation, and induces vitamin E deficiency. Zhang et al. [43] reported that treatment with dehydroepiandrosterone or melatonin, alone or in combination, prevented the reduction of B- and T-cell proliferation and the Th1 cytokine secretion caused by this retroviral infection. These hormones also suppressed the elevated production of Th2 cytokines, reduced hepatic lipid peroxidation, and prevented the loss of vitamin E.

VEE virus infection and melatonin

Melatonin administration protects mice infected with the VEE virus. When evaluated 6 days after virus inoculation (10 LD_{50} per mouse) the mortality rates of mice treated with 250, 500, and 1000 μg melatonin per kilogram were 45, 40, and 16%, respectively, as compared with 100% in control-infected mice. In addition, melatonin treatment delayed the onset of the disease and prolonged the time to death in the treated mice. In surviving mice receiving the indoleamine, the VEE virus IgM antibody titers were highly elevated 7 wk after virus inoculation [44]. The administration of melatonin significantly decreased the virus levels in blood and brain as compared with the infected control mice. When mice were pretreated with melatonin for 10 days, a significant increase in the survival rate (73%) was observed when compared with the 60% obtained after the standard 3 days of pretreatment. In cultures of chicken embryo fibroblasts with serial dilutions of VEE virus, melatonin significantly reduced the degree of cell destruction. This finding contrasts with that reported by Ben-Nathan et al. [41] who found no effect of the indoleamine on SFV growth in tissue culture. However, our results do not preclude the possibility that melatonin alters the host resistance to the virus, rather than viral replication, via a peripheral immunostimulatory effect, as fibroblasts are capable of producing tumor necrosis factor-alpha (TNF-α) whose antiviral activity is known [45]. Melatonin did not stimulate the synthesis of interleukin-2 (IL-2) but increased the serum concentration of interferon-γ (IFN-γ). In infected mice, treated or non-treated with melatonin,

IFN-γ levels in serum were also augmented. A direct and/or an immune-based effect of melatonin on VEE viral replication within the brain was suggested by the fact that on day 5 after inoculation, the virus was not detected in the brain [44].

Melatonin also prolonged the survival of immunodepressed mice infected with the VEE virus [46]. This protection is approximately 50% of that provided to immunocompetent mice suggesting that this indoleamine requires, at least partially, the integrity of the immune system to induce its protective activity. The lack of effect of melatonin on the viral titers in the brain of immunodepressed mice contrasts with the finding that melatonin administration reduces VEE virus levels in the brain of immunocompetent mice [44] suggesting that the antiviral mechanisms stimulated by melatonin in immunocompetent mice are absent or diminished in the immunodepressed animals. The protection provided by melatonin against oxidative damage to cell components [6] could be responsible, at least in part, for the increase in the survival rate of the immunodepressed infected mice. In this regard, it was demonstrated that mice with targeted deletions in either their interferon-alpha-beta-receptor (IFNAR-1-/-) or interferon regulatory factor 2 (IRF-2-/-) genes were more susceptible than control mice to VEE infection. The IFNAR-1-/- mice exhibited accelerated VEE virus dissemination to serum and brain when compared with control mice. In IRF-2-/- mice inducible nitric oxide synthetase (iNOS) gene, induction was completely absent following VEE virus infection. When the role of cells involved in iNOS production was evaluated, it was found that primary microglial cell cultures were highly sensitive to VEE virus infection. Besides, this infection increased the levels of nitric oxide in resting microglial cultures, but decreased nitric oxide production in IFN-γ-stimulated microglia. These findings suggest that reactive nitrogen species play an important role in the spread of the VEE virus [47]. It is interesting to note that melatonin inhibits cerebellar NOS activity [48] and is a potent scavenger of nitric oxide in vitro [49]. In cultured murine macrophages, melatonin has been found to inhibit nitric oxide production due to a reduction in iNOS steady-state mRNA levels and iNOS protein expression [50].

In a recent study [51], it was found that melatonin stimulated the endogenous production of IFN-γ, IL-1β, and TNF-α but not of IL-2 and IL-4 in mice treated. It has been suggested that melatonin and IFN-γ create an immunoregulatory circuit responsible for the antiviral, antiproliferative, and immunomodulatory action of IFN-γ [52]. This cytokine increases serotonin and melatonin levels in lymphocytes and macrophages [53]. The early stimulation in the production of IFN-γ by melatonin shown by Valero et al. [51] suggests that previous treatment with this indoleamine increases the antiviral activity of IFN-γ, that could possibly control viral replication at the moment of the VEE virus inoculation.

In a mouse model, the kinetics of cytokine expression following infection with molecularly cloned VEE virus showed that there is a consistent gene expression pattern associated with a rapid increase of IFN-γ, IL-6, IL-10, IL-12, and TNF-α in the absence of elevations in either IL-2 or IL-4 during responses to both attenuated and virulent VEE virus [54]. The report of Valero et al. [51] corroborates and extends some of these findings.

Interleukin-1β and TNF-α appear to be more sensitive to melatonin administration than the other cytokines assayed. Both of them were elevated earlier, immediately after treatment, and during the VEE viral infection. Melatonin triggered the host IL-1β response, suggesting an important role in the primary defense against the VEE virus infection [51]. These results are in agreement with the demonstration that this indoleamine activates monocytes and induces their cytotoxic properties, along with the IL-1β secretion [55]. The monocytes/macrophages are not only sensitive to an external stimulation by melatonin, but they also synthesize it [52]; therefore, this agent increases the reactivity of monocytes against infections.

Although melatonin increased serum levels of TNF-α and IFN-γ, the average mortality obtained, during the neutralization experiments with the corresponding anti-cytokine antibodies, suggests that neither TNF-α nor IFN-γ are essential for the protective effect of melatonin observed in murine VEE virus infection [44, 51]. The fact that 100% of the infected mice treated with melatonin died when IL-1β was blocked with antimurine IL-1β antibodies, suggests that IL-1β induced by melatonin treatment is a target cytokine to promote the immune-enhanced state which produces the viral clearance, or help generate an earlier immune response against the VEE virus infection. The study of mice lacking functional genes for IL-1β or treatment of VEE-infected mice with IL-1β should provide further insight into its role in this infection.

The survival rate of mice infected with the VEE virus preincubated with melatonin, as well as the results of the studies in vitro with the same virus, suggest that this agent does not exert a direct effect on the VEE virus [51]. In fact, it has been suggested that the encephalitic phenomenon is due more to an increased immune response in the central nervous system (CNS) than to the VEE virus replication [56].

The VEE virus infection also produced a striking increase in the levels of TNF-α and IL-1β in the brain of mice. Melatonin stimulated the endogenous production of IL-1β and reduced the concentration of TNF-α in the brain of these infected mice [57]. Elevations of IL-β levels in the brain have been detected in several viral infections of the CNS, such as those produced by the human immunodeficiency virus [58], simian immunodeficiency virus [59], SFV [60], rabies virus [61], and Sindbis virus [62]. However, these studies did not directly examine whether IL-1β plays a protective or deleterious role in CNS viral infections. Liang et al. [63] compared the natural histories of deficient and wild-type 129 SV (ev) mice infected with a neurovirulent viral strain, neuroadapted Sindbis virus. Their results suggest that IL-1β deficiency is protective against fatal Sindbis virus infection. In contrast, mice deficient in IL-1β have increased susceptibility to influenza virus [64], and in poxvirus animal models the viral induction of this cytokine is also beneficial for the host [65].

The increase in IL-1β levels detected in blood [51] and brain [57] of VEE virus-infected mice after melatonin

treatment plays a protective role to the infected animals, as shown by the effects of the blockade of IL-1β with antimurine IL-1β antibodies [51]. The mechanism of this effect is unknown, but it is possible to envision that during the VEE viral infection certain degenerating neurons that contain substance P might release this neuropeptide, which subsequently induces the release of IL-1β and TNF-α from monocytes [66], which can then infiltrate the CNS from the blood [67]. Microglia proliferate and phagocytose local neuronal debris, resulting in microglial synthesis of IL-1β [67, 68], which in turn induces the expression of adhesion molecules on local endothelium, favoring transmigration of lymphocytes and monocytes [69]. The increase in IL-1β levels produced by melatonin would provide neuronal support and protection by inducing nerve growth factor secretion by astrocytes [70], thereby supplying a trophic factor for many neuronal cell types in times of stress, such as that produced by the VEE virus infection.

TNF-α seems to be upregulated in a wide range of CNS disorders [71, 72] and has been implicated in both neuronal death and survival [73–76]. It plays an important role in the initiation of inflammation and the resulting pathological effects and can stimulate different cell types to produce several cytokines including IL-1β and TNF-α itself [77, 78].

In VEE virus infection it is presumed that upon stimulation by the increased brain levels of TNF-α, astrocytes produce colony-stimulating factors that can augment inflammatory responses because of their leukocyte chemotactic properties, which would promote migration of granulocytes and macrophages to inflammatory sites within the CNS [79]. Astrocyte-derived TNF-α can induce intercellular adhesion molecules on neighboring endothelial cells [80], alter blood-brain barrier permeability, and promote inflammatory infiltration into the CNS [81]. Therefore, it is reasonable to assume that the highly significant decrease in the concentration of brain TNF-α produced by melatonin in mice infected with the VEE virus [57] is aimed at diminishing the inflammatory response in the CNS and at correcting the alteration in blood-brain barrier permeability. Thus, the indoleamine would help to protect the brain of the infected mice.

High intensity light and VEE virus infection

In VEE-infected mice exposed to a high intensity light (HIL) of 2500 lux with a 12 h light:12 h dark photoperiod, both nocturnal and diurnal serum levels of melatonin were constantly elevated and the survival rate increased from 6 to 13 days after virus inoculation. Melatonin concentrations remained high throughout the day and night, breaking the established pattern observed in the 400 lux control mice, characterized by high levels of melatonin at night and low levels during the day [82]. These results confirm that the intensity of illumination during the light period affects the metabolism of pineal indoles in rodents [83]. The HIL seems to be responsible for the rise in melatonin production and the associated increase in the survival rate of the infected mice.

Exposure to 2500 lux also increased significantly the levels of melatonin in the olfactory bulb of VEE-infected mice [84]. This rise in melatonin might be one of the mechanisms of defense against the viral attack as VEE virus enters the brain first via olfactory pathways [85].

Melatonin enhances immunization against VEE virus

The immunoenhancing properties of melatonin treatment were evident in mice vaccinated with the VEE virus TC-83. Blood IgM titers in mice treated with multiple doses of melatonin (1 mg/kg) were significantly higher than those of the control group, at day 14 after vaccination. When treated with multiple doses of melatonin (5 mg/kg) the antibody titers rose significantly after the first week of immunization [86]. It is known that IL-10 induces activated B cells to secrete large amounts of IgA, IgG, and IgM antibodies [87]. The increase in IL-10 serum levels detected in mice vaccinated with TC-83 and treated with 1 and 5 mg/kg of melatonin, coincided with the rise in antibody titers displayed by these groups of mice at weeks 1 and 2 postimmunization. However, the virus levels in the brains of mice challenged with live VEE virus at day 21 postimmunization were significantly reduced. These results suggest that melatonin treatment enhanced the efficiency of mice immunization against VEE virus [86].

In conclusion, melatonin seems to have an immune effect in the VEE viral infection, probably mediated by IL-1β. This interleukin has been considered one of the earliest host mediators during infectious diseases of the CNS [88]. In these studies, the immunomodulatory properties of melatonin are evident and suggest this indoleamine as a therapeutic alternative to resist VEE and other viral diseases, including SARS as no antimicrobial agent has been proven to be effective for its treatment. The use of melatonin to treat SARS has been suggested by Shiu et al. [89].

References

1. BINKLEY SA. Circadian rhythms of pineal function in rats. Endocr Rev 1983; **4**:255–270.
2. LIEBERMAN HR, WALDHAUSER F, GARFIELD G et al. Effects of melatonin on human mood and performance. Brain Res 1984; **323**:201–207.
3. REITER RJ. The mammalian pineal gland: structure and function. Am J Anat 1981; **162**:287–313.
4. KRAUSE DN, DUBOCOVICH ML. Regulatory sites in the melatonin system of mammals. TINS 1990; **13**:464–470.
5. AMSTRONG SM. Entrainment of vertebrate circadian rhythms by melatonin. Adv Pineal Res 1991; **5**:259–266.
6. REITER RJ, MELCHIORRI D, SEWERYNEK E et al. A review of the evidence supporting melatonin's role as an antioxidant. J Pineal Res 1995; **18**:1–11.
7. PIERI C, MARRA M, MORONI F et al. Melatonin: a peroxyl radical scavenger more effective than vitamin E. Life Sci 1994; **59**:PL271–PL276.
8. REITER RJ. Functional aspects of the pineal hormone melatonin in combating cell and tissue damage induced by free radicals. Eur J Endocrinol 1996; **134**:412–420.
9. MAESTRONI GJM. The immunotherapeutic potential of melatonin. Exp Opin Invest Drugs 2001; **10**:467–476.

10. MAESTRONI GJM. T-helper 2 lymphocytes as peripheral target of melatonin signaling. J Pineal Res 1995; **18**:84–89.

11. CURRIER NL, SUN LZ, MILLER SC. Exogenous melatonin: quantitative enhancement in vivo of cells mediating non-specific immunity. J Neuroimmunol 2000; **104**:101–108.

12. LISSONI P, BARNI S, TANCINI G et al. A study of the mechanism involved in the immunostimulatory action of the pineal hormone in cancer patients. Oncology 1993; **50**:399–402.

13. MAESTRONI GJM, CONTI A, PIERPAOLI W. Role of the pineal gland in immunity. Circadian synthesis and release of melatonin modulates the antibodies response and antagonizes the immunosuppressive effect of corticosterone. J Neuroimmunol 1986; **13**:19–30.

14. MAESTRONI GJM, CONTI A, PIERPAOLI W. Role of the pineal gland in immunity: II. Melatonin enhances the antibody response via an opiatergic mechanism. Clin Exp Immunol 1987; **68**:384–391.

15. CARDINALI DP, GOLOMBEK DA, ROSENSTEIN RE et al. Melatonin site and mechanism of action: single or multiple. J Pineal Res 1997; **23**:32–39.

16. DUBOCOVICH ML. Melatonin receptors: are there multiple subtypes? Trends Pharmacol Sci 1995; **16**:50–56.

17. REPPERT SM, WEAVER DR. Melatonin madness. Cell 1995; **83**:1059–1062.

18. CALVO JR, RAFII-EL-IDRISSI M, POZO D et al. Immunomodulatory role of melatonin: specific binding sites in human and rodent lymphoid cells. J Pineal Res 1995; **18**:119–126.

19. GUERRERO JM, REITER RJ. Melatonin immune system relationships. Curr Top Med Chem 2002; **2**:167–179.

20. LOPEZ-GONZALEZ MA, CALVO JR, OSUNA C et al. Interaction of melatonin with human lymphocytes: evidence for binding sites coupled to potentiation of cyclic AMP stimulation by vasoactive intestinal peptide and activation of cyclic GMP production. J Pineal Res 1992; **12**:97–104.

21. BARJAVEL MJ, MAMDOUH Z, RAGHBATE N et al. Differential expression of the melatonin receptor in human monocytes. J Immunol 1998; **160**:1191–1197.

22. LOPEZ-GONZALEZ MA, CALVO JR, SEGURA JJ et al. Characterization of MEL binding sites in human peripheral blood neutrophils. Biotech Ther 1993; **4**:253–262.

23. GARCIA-PERGAÑEDA A, POZO D, GUERRERO JM et al. Signal transduction for melatonin in human lymphocytes: involvement of a pertussis-toxin sensitive G protein. J Immunol 1997; **159**:3774–3781.

24. LIU ZM, PANG SF. [^{125}I]iodomelatonin binding sites in the bursa of Fabricius of birds: binding characteristics, subcellular distribution, diurnal variation and age studies. J Endocrinol 1993; **138**:51–57.

25. GARCIA-MAURIÑO S, GONZALEZ-HABA MG, CALVO JR et al. Involvement of nuclear binding sites for melatonin in the regulation of IL-2 and IL-6 production by human blood mononuclear cells. J Neuroimmunol 1998; **92**:76–84.

26. RAFII-EL-IDRISSI M, CALVO JR, HARMOUCH A. Specific binding of melatonin by purified cell nuclei from spleen and thymus of the rat. J Neuroimmunology 1998; **86**:190–197.

27. CALISHER CH, KARABATSOS N. Arbovirus serogroups: definition and geographic distribution. In: The Arboviruses: Epidemiology and Ecology. Monath TP, ed. CRC Press, Boca Raton, 1988; pp. 19–57.

28. SOTO ESCALONA A, FINOL LT, RYDER S. Estudio de un brote de encefalitis venezolana en el Distrito Paez, Estado Zulia, en Octubre de 1968. Invest Clin 1969; **10**:45–47.

29. NEGRETTE A, MOSQUERA J. 1959 en Maracaibo (San Francisco), Estado Zulia, Venezuela. Manifestaciones clínicas y terapeutica antibiotica. Invest Clin 1974; **15**:11–44.

30. BOWEN GS, CALISHER CH. Virological and serological studies of Venezuelan equine encephalomyelitis in humans. J Clin Microbiol 1976; **4**:22–27.

31. BONILLA E, PRASAD ALN, ESTEVEZ J et al. Changes in serum and striatal free amino acids after Venezuelan equine encephalomyelitis virus. Exp Neurol 1988; **99**:647–654.

32. WEAVER SC, SALAS R, RICO-HESSE R et al. Reemergence of epidemic Venezuelan equine encephalitis in South America. Lancet 1996; **348**:436–440.

33. TSAI TF, POPOVICI F, CERNESCU C. West Nile encephalitis epidemic in southeastern Romania. Lancet 1998; **352**:767–771.

34. WILLEMS WR, KALUZA G, BOSCHERK CB et al. Semliki Forest virus: cause of a fatal case of human encephalitis. Science 1979; **203**:1127–1129.

35. BRADISH CJ, ALLNER K, MABER HB. The virulence of original and derived strains of Semliki Forest virus for mice, guinea pigs, and rabbits. J Gen Virol 1971; **12**:141–160.

36. KSIAZEK TG, ERDMAN D, GOLDSMITH CS et al. A novel coronavirus associated with Severe Acute Respiratory Syndrome. N Engl J Med 2003; **348**:1953–1965.

37. VU TH, CABAU JF, NGUYEN NT et al. SARS in Northern Vietnam. N Engl J Med 2003; **348**:20–35.

38. RAGER-ZISMAN B, ALLISON AC. The role of antibodies and host cells in the resistance of mice against infection by Coxsakie B3 virus. J Gen Virol 1973; **19**:329–338.

39. THONG HY, VINCENT MM, HENSEN SA et al. Depressed specific cell mediated immunity to herpes simplex virus type 1 in patients with recurrent herpes labialis. Infect Immun 1975; **12**:76–80.

40. MAESTRONI GJM, CONTI A, PIERPAOLI W. Pineal melatonin, its fundamental immuno-regulatory role in aging and cancer. Ann N Y Acad Sci 1988; **521**:140–148.

41. BEN-NATHAN D, MAESTRONI GJM, LUSTIG S et al. Protective effects of melatonin in mice infected with encephalitis viruses. Arch Virol 1995; **140**:223–230.

42. ELLIS LC. Melatonin reduces mortality from Aleutian disease in mink (Mustela vison). J Pineal Res 1996; **21**:214–217.

43. ZHANG Z, ARAGHI-NIKNAM M, LIANG B et al. Prevention of immune dysfunction and vitamin E loss by dehydroepiandrosterone and melatonin supplementation during murine retrovirus infection. Immunology 1999; **96**:291–297.

44. BONILLA E, VALERO N, PONS H et al. Melatonin protects mice infected with Venezuelan equine encephalomyelitis virus. Cell Mol Life Sci 1997; **53**:430–434.

45. GUIDOTTI L, CHISARI FV. To kill or to cure: options in host defense against viral infection. Curr Opin Immunol 1996; **8**:478–483.

46. BONILLA E, RODON C, VALERO N et al. Melatonin prolongs survival of immunodepressed mice infected with the Venezuelan equine encephalomyelitis virus. Trans R Soc Trop Med Hyg 2001; **95**:207–210.

47. SCHONEBOOM BA, LEE JS, GRIEDER FB. Early expression of IFN-alpha/beta and iNOS in the brains of Venezuelan equine encephalitis virus-infected mice. J Interferon Cytokine Res 2000; **20**:205–215.

48. POZO D, REITER RJ, CALVO JR et al. Inhibition of cerebellar nitric oxide synthase and cyclic GMP production by melatonin via complex formation with calmodulin. J Cell Biochem 1997; **65**:430–442.

49. NODA Y, MORI A, LIBURDY R et al. Melatonin and its precursors scavenge nitric oxide. J Pineal Res 1999; **27**:159–163.

50. GILAD E, WONG HR, ZINGARELLI B et al. Melatonin inhibits expression of the inducible isoform of nitric oxide synthase in murine macrophages: role of inhibition of NFKappaB activation. FASEB J 1998; **12**:685–693.

51. VALERO N, BONILLA E, PONS H et al. Melatonin induces changes to serum cytokines in mice infected with the Venezuelan equine encephalomyelitis virus. Trans R Soc Trop Med Hyg 2002; **96**:1–4.

52. FINOCCHIARO LM, ARZT ES, FERNANDEZ S et al. Serotonin and melatonin synthesis in peripheral blood mononuclear cells: stimulation by interferon-gamma as part of the immunomodulatory pathway. J Interferon Res 1988; **8**:705–716.

53. ARZT E, FERNANDEZ S, FINOCCHIARO L et al. Immunomodulation by indoleamines: serotonin and melatonin action on DNA and interferon-gamma synthesis by human peripheral blood mononuclear cells. J Clin Immunol 1988; **8**:513–520.

54. GRIEDER F, DAVIS H, ZHOU X et al. Kinetics of cytokine expression and regulation of host protection following infection with molecularly cloned Venezuelan equine encephalomyelitis virus. Virology 1997; **233**:302–312.

55. MORREY K, MCLACHLAN A, SERKIN C et al. Activation of human monocytes by the pineal hormone melatonin. J Immunol 1994; **153**:2671–2680.

56. CHARLES P, WALTER E, MARGOLIS F et al. Mechanism of neuroinvasion of Venezuelan equine encephalitis virus in the mouse. Virology 1995; **208**:662–671.

57. BONILLA E, VALERO N, CHACÍN-BONILLA L et al. Melatonin increases interleukin-1β and decreases tumor necrosis factor alpha in the brain of mice infected with the Venezuelan equine encephalomyelitis virus. Neurochem Res 2003; **28**:687–692.

58. GRIFFIN DE. Cytokines in the brain during viral infection: clues to HIV-associated dementia. J Clin Invest 1997; **100**:2948–2951.

59. LANE TE, BUCMEIER MJ, WATRY DD et al. Expression of inflammatory cytokines and inducible nitric oxide synthase in brains of SIV-infected rhesus monkeys: applications to HIV-induced central nervous system disease. Mol Med 1996; **1**:27–37.

60. MOKHTARIAN F, WESSELING SL, CHOI S et al. Production and role of cytokines in the CNS of mice with acute viral encephalomyelitis. J Neuroimmunol 1996; **66**:11–22.

61. MARQUETTE C, VANDAM AM, CECCALDI PE et al. Induction of immunoreactive interleukin-1 beta and tumor necrosis factor-alpha in the brains of rabies virus infected rats. J Neuroimmunol 1996; **68**:45–51.

62. WESSELING SL, LEVINE B, FOX RJ et al. Intracerebral cytokine mRNA expression during fatal and nonfatal alpha virus encephalitis suggests a predominant type 2 T cell response. J Immunol 1994; **152**:1289–1297.

63. LIANG XH, GOLDMAN JE, JIANG HH et al. Resistance of interleukin-1β-deficient mice to fatal Sindbis virus encephalitis. J Virol 1999; **73**:2563–2567.

64. KOZAK W. Thermal and behavioural effects of lipopolysaccharide and influenza in interleukin-1 beta deficient mice. Am J Physiol 1995; **269**:R969–R977.

65. SPRIGGS MK, HRUBY DE, MALISZEWSKI CR et al. Vaccinia and cowpox viruses encode a novel secreted interleukin-1-binding protein. Cell 1992; **71**:145–152.

66. LOTZ M, VAUGHAN JH, CARSON DA. Effect of neuropeptides on production of inflammatory cytokines by human monocytes. Science 1988; **241**:1218–1221.

67. PERRY VH, ANDERSON PB, GORDON S. Macrophages and inflammation in the central nervous system. TINS 1993; **16**:268–273.

68. GIULIAN D, LI J, LEARA B et al. Phagocytic microglia release cytokines and cytotoxins that regulate the survival of astrocytes and neurons in culture. Neurochem Int 1994; **25**:227–233.

69. FABRY Z, RAINE CS, HART MN. Nervous tissue as an immune compartment: the dialect of the immune response in the CNS. Immunol Today 1994; **15**:218–224.

70. CARMAN-KRZAN M, WISE BC. Arachidonic acid lipoxygenation may mediate interleukin-1 stimulation of nerve growth factor secretion in astroglial cultures. J Neurosci Res 1993; **34**:225–232.

71. PROBERT L, AKASSOGLOU K, PASPARAKIS M et al. Spontaneous inflammatory demyelinating disease in transgenic mice showing central nervous system specific expression of tumor necrosis factor α. Proc Natl Acad Sci USA 1995; **92**:11294–11298.

72. CHOE W, STOLCA G, LYNN W et al. Neurodegeneration induced by MoMuLV-ts 1 and increased expression of Fas and TNF-alpha in the central nervous system. Brain Res 1998; **779**:1–8.

73. BEATTIE MS, FAROOQUI A, BRESNAHAN JC. Review of current evidence for apoptosis after spinal cord injury. J Neurotrauma 2000; **17**:915–925.

74. BEATTIE EC, STELLWAGEN D, MORISHITA W et al. Control of synaptic strength by glial TNF-α. Science 2002; **295**:2282–2285.

75. ALLAN SM, ROTHWELL NJ. Cytokine and acute neurodegeneration. Nat Rev Neurosci 2001; **2**:734–744.

76. YANG L, LINDHOLM K, KONISHI Y et al. Target depletion of distinct tumor necrosis factor receptor subtypes reveals hippocampal neuron death and survival through different signal transduction pathways. J Neurosci 2002; **22**:3025–3032.

77. TRACEY KJ, CERAMI A. Tumor necrosis factor, other cytokines and disease. Ann Rev Cell Biol 1993; **9**:317–343.

78. TRACEY KJ, CERAMI A. Tumor necrosis factor: an updated review of its biology. Crit Care Med 1993; **21**:S415–S422.

79. TWEARDY DJ, GLAZER EW, MOTT PL et al. Modulation by tumor necrosis factor-α of human astroglial cell production of granulocyte-macrophage colony-stimulating factor (G-CSF). J Neuroimmunol 1991; **32**:269–278.

80. MCCARRON RM, WANG L, RACKE MK et al. Cytokine-regulated adhesion between encephalitogenic T lymphocytes and cerebrovascular endothelial cells. J Neuroimmunol 1993; **43**:23–30.

81. BENVENISTE EN, BENOS DJ. TNF-α and IFN-α mediated signal transduction pathways: effects on glial cell genes expression and function. FASEB J 1995; **9**:1577–1584.

82. MEDINA S, VALERO-FUENMAYOR N, CHACIN-BONILLA L et al. Exposure to 2500 lux increases serum melatonin in Venezuelan equine encephalomyelitis. Neurochem Res 1999; **24**:775–778.

83. AZUMA H, YAMATODANI A, YAGI A et al. Influence of intensity of illumination during the light period on diurnal variations of pineal indoles in rats and mice. Neurosci Lett 1990; **119**:15–18.

84. MEDINA-LEENDERTZ S, VALERO N, CHACIN-BONILLA L et al. High intensity light increases olfactory bulb melatonin in Venezuelan equine encephalomyelitis virus infection. Neurochem Res 2001; **26**:231–234.

85. VOGEL P, ABPLANALP D, KELL W et al. Venezuelan equine encephalitis in BALB/c mice: kinetic analysis of central nervous system infection following aerosol or subcutaneous inoculation. Arch Pathol Lab Med 1996; **120**:164–172.

86. NEGRETTE B, BONILLA E, VALERO N et al. Melatonin treatment enhances the efficiency of mice immunization with Venezuelan equine encephalomyelitis virus TC-83. Neurochem Res 2001; **26**:765–768.

87. ROUSSET F, GARCIA E, DE FRANCE T et al. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc Natl Acad Sci USA 1992; **89**:1890–1893.

88. BLATTEIS C. Pyrogenic action of cytokines. In: Interleukin-1 in the Brain. Rothwell N, Dantzer R, eds. Pergamon Press, Oxford, 1992; pp. 93–114.

89. SHIU SYW, REITER RJ, TAN DX et al. Urgent search for safe and effective treatments of severe acute respiratory symdrome: is melatonin a promising candidate drug. J Pineal Res 2003; **35**:69–70.