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REVIEW

Beneficial actions of melatonin in the management of viral infections: a new use for this "molecular handyman"?

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SUMMARY

Melatonin (N-acetyl-5-methoxytryptamine) is a multifunctional signaling molecule that has a variety of important functions. Numerous clinical trials have examined the therapeutic usefulness of melatonin in different fields of medicine. Clinical trials have shown that melatonin is efficient in preventing cell damage under acute (sepsis, asphyxia in newborns) and chronic states (metabolic and neurodegenerative diseases, cancer, inflammation, aging). The beneficial effects of melatonin can be explained by its properties as a potent antioxidant and antioxidant enzyme inducer, a regulator of apoptosis and a stimulator of immune functions. These effects support the use of melatonin in viral infections, which are often associated with inflammatory injury and increases in oxidative stress. In fact, melatonin has been used recently to treat several viral infections, which are summarized in this review. The role of melatonin in infections is also discussed herein. Copyright © 2012 John Wiley & Sons, Ltd.

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Abbreviations

ALRs, AIM2-like receptors; AMDV, Aleutian mink disease virus; AP-1, activating protein-1; ATF-2, activation transcription factor 2; BBB, blood–brain barrier; CAT, catalase; DISC, death-inducing signaling complex; EMCV, encephalomyocarditis virus; GM-CSF, granulocyte-macrophage colony-stimulating factor; GPx, glutathione peroxidase; GST, glutathione-s-transferase; HPV, human papillomavirus; IFIT, interferon-induced protein with tetratricopeptide; iNOS, inducible NO synthase; IRF3, interferon regulatory factor 3; IRF7, interferon regulatory factor 7; ISG, interferon-stimulated genes; JNK, Janus kinase; MCP-1, monocyte chemotactic protein-1; MDA, malondialdehyde; MLV, murine leukemia virus; mtPTP, mitochondrial permeability transition pore; NF-κB, nuclear factor kappa B; NK, natural killer cells; NKT cells, natural killer T cells; NLRs, Nod-like receptors; Nrf2, nuclear factor erythroid 2; OAS, oligoadenylate synthetases; PAMPs, pathogen-associated molecular patterns; PCD, programmed cell death; pDC, plasmacytoid dendritic cells; PKR, dsRNA-activated protein kinase; PRRs, pattern recognition receptors; RANTES, regulated upon activation, normal T cell expressed and secreted; RHDV, rabbit hemorrhagic disease virus; RLRs, RIG-I-like receptors; SeV, Sendai virus; SFV, Semliki Forest virus; SOD, superoxide dismutases; TBE-V, tickborn encephalitis virus; TGFβ, transforming growth factor-β; Th1, type 1 T helper cell; Th2, type 2 T helper cell; TLRs, Toll-like receptors; TNF-R, tumor necrosis factor receptor; VEE, Venezuelan equine encephalomyelitis; VEEV, Venezuelan equine encephalomyelitis virus; VSV, vesicular stomatitis virus; WNV, West Nile virus; XO, xanthine oxidase.

INTRODUCTION

The methoxyindole melatonin (N-acetyl-5-methoxytryptamine) is a secretory product of the pineal gland. It was first reported as a skin lightening agent in amphibians [1,2]. Further investigations showed that another function, supported by its direct effects in regions containing high densities of melatonin receptors, such as the circadian pacemaker (the suprachiasmatic nucleus) and the pars tuberalis, is to regulate and reset circadian rhythms as well as to be involved in the measurement of day length, an environmental variable used for seasonal timing of reproduction, metabolism and behavior in species responding to photoperiodic changes [3–7].

In recent decades, melatonin has been reported to possess numerous additional functions and act in neural and non-neural tissues or cells that express melatonin receptors that are at lower densities than in the suprachiasmatic nucleus. Thus, melatonin is involved in sleep initiation, vasomotor control, anti-excitatory actions, immunomodulation including possessing anti-inflammatory properties, antioxidant actions, and actions on energy

metabolism, influences on mitochondrial electron flux, regulation of the mitochondrial permeability transition pore (mtPTP), and mitochondrial protection against free radicals [8–13]. Deficiencies in melatonin production or melatonin receptor expression and decreases in melatonin levels (such as those that occur during aging) are likely to contribute to numerous dysfunctions [14–16]. In fact, several clinical trials have shown that melatonin is efficient in preventing cell damage under acute (sepsis, asphyxia in newborns) and chronic states (metabolic and neurodegenerative diseases, cancer, inflammation, aging) [17–22]. In humans, the efficacy of melatonin as a treatment of ocular diseases, cardiovascular diseases, sleep disturbances and several other pathologies, as well as a complementary treatment in anesthesia, haemodialysis, in vitro fertilization and neonatal care, has been assessed and reported to be beneficial [23]. Likewise, melatonin reduces the toxicity and increases the efficacy of a large number of drugs whose side effects are well documented [24].

The beneficial effects of melatonin are explained by its properties as a potent antioxidant, a modulator of apoptosis and a positive regulator of immune functions [25–29]. These actions suggest the potential to treat viral infections, which usually cause inflammatory injury and elevated oxidative stress [30,31]. A number of reports examining the ability of melatonin to protect against viral infections have been published, as summarized in the following section.

FIRST EVIDENCE RELATED TO THE ABILITY OF MELATONIN TO PROTECT AGAINST TO VIRAL INFECTIONS

Encephalomyocarditis virus (EMCV) is a highly pathogenic and aggressive virus that causes encephalitis and myocarditis in rodents. Administration of melatonin prevented paralysis and death of mice infected with sublethal doses of EMCV [32]. Melatonin also has a protective effect in mice infected with Semliki Forest virus (SFV), a classic encephalitis arbovirus, that invades the CNS and whose replication in the mouse brain eventually leads to death. Melatonin administration not only reduced the death rate but also significantly postponed the onset of the disease. Furthermore, the level of virus in the blood in melatonin-treated mice was lower than in non-treated mice [33]. Although attenuated West Nile virus (WNV) strain WN-25 is an encephalitis virus that does not invade the brain and does not normally cause encephalitis, exposure of mice to various stressful stimuli induces WN-25 encephalitis. Melatonin counteracts the immunodepressive effect of stress exposure and prevents the stress-related encephalitis and death of WN-25 infected mice [33].

Venezuelan equine encephalomyelitis (VEE) is an important human and equine disease caused by VEE virus (VEEV), a mosquito-borne organism. Outbreaks have occurred in northern South America from the 1920s to the 1970s with thousands of people and horses, donkeys and related species being infected. Mice have been used as an animal model for this condition, because VEEV-infected mice show excitation and hypermotility followed by hypomotility, paralysis, coma and death. Melatonin administration protects mice infected with VEEV by decreasing the virus load in brain and serum, reducing mortality rates, delaying the onset of the disease and deferring the time to death. Furthermore, in surviving mice treated with melatonin, the VEEV-mediated IgM antibody titres are highly elevated [34].

Aleutian mink disease is a natural condition caused by persistent infection with the Aleutian mink disease virus (AMDV). Animals in the progressive state of the disease show a marked hypergammaglobulinemia, because of high titers of non-neutralizing ADMV antibodies. This is thought to cause lesions in the kidney, liver, lungs and arteries. Melatonin implants reduced mortality in ADMV-infected mink [35].

The findings in these reports document the ability of the melatonin to protect against viral infections [Table 1]. The potential protective mechanisms include melatonin acting as a free radical scavenger, an antioxidant enzyme inducer, a positive regulator of immune functions and an inhibitor of inflammation, as well as a regulator of programmed cell death (PCD) [Table 2].

MELATONIN AS A FREE RADICAL SCAVENGER AND ANTIOXIDANT ENZYME INDUCER IN VIRAL INFECTIONS

Free radicals are molecules formed naturally during many metabolic processes. They contain an unpaired electron in their valence orbital that makes them unstable and reactive. These reactive agents damage essential molecules in cells including lipids, proteins and DNA [36,37]. Among these

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Table 1. First evidence related to the ability of melatonin to protect against viral infections

Virus	Animals	Doses of melatonin	Effects	Ref.
EMCV	25 female 2-3-months-old BALB/cj mice	1 μg i.p./mouse daily for 10 days	Prevention of paralysis and death of infected mice	32
SFV	18 Charles River outbred ICR female mice (CD1)	500 μg/kg s.c. daily, 3 days before until 10 days after virus inoculation	Reduction of the death rate	33
			Delay of the onset of the disease	
	10 Charles River outbred ICR female mice (CD1)	500 μg/kg s.c. daily, 3 days before until 10 days after virus inoculation	Decrease the virus load in blood	33
WN-25*	16 Charles River outbred ICR female mice (CD1)	5 μg/mouse s.c. daily, 2 days before until 8 days after virus inoculation	Counteracts the immunodepressive effect of stress exposure Prevention of the stress-related encephalitis Prevention of the death of infected mice	33
VEEV	25 male albino mice (NMRI- IVIC strain)/group	250–500 μg–1 mg/kg s.c. daily, 3 days before until 10 days after inoculation	Reduction of mortality rates	34
			Delay of the onset of the disease Deferring of the time to death	
	6 male albino mice (NMRI- IVIC strain)	500 μg/kg s.c. daily, 3 days before until 10 days after inoculation	Decrease the virus load in brain Decrease the virus load in serum	34
	3 male albino mice (NMRI- IVIC strain)/group	250–500 μg/kg s.c. daily, 3 days before until 10 days after inoculation	Increase the VEEV-mediated IgM antibody titres	34
AMDV	90 wild type (demi-buff or demi strain) minks 6000 male and female demi strain minks 3000 male and female demi and mahogany strains of kit minks	Silastic implants (0.65 cm length, 0.21 cm diameter) containing 2.7 mg melatonin crystals homogeneously suspended in medical grade silastic polymer	Reduction of mortality rates	35

EMCV, encephalomyocarditis virus; SFV, Semliki Forest virus; VEEV, Venezuelan equine encephalomyelitis virus; AMDV, Aleutian mink disease virus; i.p., intraperitoneal; s.c., subcutaneal.
*an atenuated West Nile virus strain

reactants, the superoxide anion radical ($O_2{\bullet}^-$), nitric oxide (NO•) and especially their derivatives, the hydroxyl radical (•OH) and peroxynitrite ($ONOO^-$), are highly biologically damaging elements produced in the host during microbial infections [38–41]. Phagocytes, such as neutrophils and macrophages are assumed to be the major generators of free radicals. Elevated levels of $O_2{\bullet}^-$ are

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Table 2. Effects of melatonin in protecting against viral infections

Properties	Virus	Animal/cell cultures	Effects of melatonin administration	Ref.
Free radical scavenger	VEEV	Murine splenocytes	Reduction of NO• concentrations in tissue	105
		Murine neuroblastoma	Decrease of both NO• and lipid peroxidation	106
		Mice	Reduces nitrite concentrations in the brain and serum Lowering lipid peroxidation products	107
	RSV	Mice	Reduction of acute lung oxidative injury Suppression of MDA, NO• and •OH generation Restoration of GSH and SOD levels in the lungs	31
Antioxidant enzyme inducer	RHDV	Rabbits	Restoration of activity and mRNA expression of GPx, GST and Mn-SOD Rise in protein expression of Nrf2	109
Regulator of immune functions	MLV	Mice	Prevention of reduction in B- and T-cell proliferation Prevention in Th1 cytokine secretion Prevention of overproduction of Th2 cytokines and TNF-α	156
	VEEV	Mice	Stimulation of endogenous production of IL-1β in brain Reduction of the concentration of TNF-α in brain	158
			Stimulation of endogenous production of IFN-γ, IL-1β, and TNF-α in serum	159
	RSV	Mouse macrophages	Decrease of TLR3-mediated downstream gene expression	170
Regulator of PCD	RHDV	Rabbits	Reduction of Bax expression Reduction of cytosolic cytochrome c release Increased expression of Bcl-2 and Bcl-xL Inhibition of caspase-9 activity Reduction in caspase-8 activity Reduction in TNF-R1 and JNK expression Increased expression of c-FLIP	179

VEEV, Venezuelan equine encephalomyelitis virus; RHDV, rabbit hemorrhagic disease virus; MLV, murine leukemia virus.

generated by both phagocyte NADPH oxidase and xanthine oxidase (XO) during viral infections [42–46]. $O_2•^-$ reduces ferric iron to ferrous iron, which catalyzes the Fenton reaction and generates •OH from hydrogen peroxide. $ONOO^-$ is formed by the coupling of $O_2•^-$ and NO•.

Overproduction of NO• is primarily caused by activation of inducible NO synthase (iNOS), which is usually expressed by inflammatory phagocytes and other cell types (e.g. epithelial and neuronal cells) [37,38,40,47]. iNOS is regulated by cytokine-dependent mechanisms in HIV-1, HBV and HCV infections [48–51], as well as in a variety of experimental viral infections in rats and mice, including neurotropicviruses (Borna disease virus, HSV-1 and rabies virus), and pneumotropic and

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cardiotropic viruses (influenza virus, SeV and coxsackie virus) [52–59].

Although IFN-γ is the major cytokine inducing iNOS and NO• overproduction in the pathogenesis of these viral infections, iNOS expression is down-regulated by IL-4, IL-10 and transforming growth factor-β (TGF-β) [60–62]. IFN-γ is known to be associated with type 1 helper T cell (Th1) responses, and IL-4 and IL-10 are induced by type 2 helper T cell (Th2) responses; NO• biosynthesis catalyzed by iNOS is precisely regulated by a polarized Th1–Th2 balance. In other viral diseases, viral replication or viral components directly induce iNOS without mediation by pro-inflammatory cytokines. Thus, the HIV envelope glycoprotein gp41 triggers iNOS expression in human astrocytes and murine cortical brain cells in culture [63,64]. RSV directly upregulates iNOS in human type 2 alveolar epithelial cells (A549 cells) [65].

Free radicals are produced to eliminate the pathogenic agent or to kill the virus-infected cells by a non-specific response. Thus, antiviral effects of NO• have been described for some DNA viruses such as murine poxvirus (ectromelia virus) and herpes viruses including HSV, EBV and some RNA viruses such as Coxsackie virus [66–71]. The toxic oxygen and nitrogen-based reactants, unfortunately, cannot discriminate between exogenous invading pathogens and the host cells themselves, and therefore, they also damage the host. To minimize such self-damage during the elimination of pathogens, the host employs several primitive tactics; it uses recruited phagocytes for the physical containment of pathogens in infectious foci. Most bacteria, for example, can be phagocytosed and confined to septic foci, which are typically abscesses or granulomas. Under these conditions, free radicals can affect bacteria rather selectively with the surrounding normal tissue remaining mostly intact.

In viral infections, in contrast, free radical mediators cause non-specific oxidative/nitrosative damage in virus-infected tissue and produce oxidative stress; this occurs when the virus cannot be confined to limited areas by the non-specific host defense [56,58,72]. Thus, NO• has appreciable antiviral actions on several types of viruses including ortho- and paramyxovirus, murine vaccinia virus, coronavirus (mouse hepatitis virus), lymphocytic choriomeningitis virus, murine EMCV, tickborn encephalitis virus (TBE-V) [73–78]; also, NO• and its derivatives, especially $ONOO^-$, can be considered pathogenic in some viral infections. Indeed, NO• inhibition or lack of NO• generation reduces the pathological consequences of viral pneumonia in mice caused by influenza virus, SeV and HSV-1, HSV-1-induced encephalitis in rats, EMCV-induced carditis and diabetes, and murine encephalitis induced by flavivirus (Murray Valley encephalitis virus, TBE-V) [55,57,74,78–82]. A similar pathogenicity with a lack of antiviral effects has been observed for $O_2•^-$ in several experimental models of virus-induced pneumonia including those caused by influenza virus and CMV [43–45,56,72,83,84].

HCV-induced oxidative stress is emerging as a key step and a major initiator in the development and the progression of liver damage [85]. NS3, one of the non-structural proteins of HCV, was reported to induce reactive oxygen species by NADPH oxidase in neutrophils [86]. High-risk human papilloma virus (HPV), which causes cervical cancer, promotes iNOS-dependent DNA damage, leading to dysplastic changes and carcinogenesis [87].

Epstein–Barr virus is a herpes virus that infects the majority of the world population, generally during childhood; it has been linked to the genesis of a number of lymphoproliferative diseases and neoplasia such as the African Burkitt lymphoma, nasopharyngeal carcinoma or gastric carcinoma. Early stages of EBV infection generate oxidative stress either in B lymphocytes or in epithelial cells, so contributing to pathology [88]. Influenza A virus causes a respiratory disease, which ranges from mild upper respiratory tract illness with or without fever to severe complications such as pneumonia. The latter disease results in respiratory failure, acute respiratory distress syndrome, multi-organ failure and even death. An abrupt increase in $O_2•^-$ production occurs during phagocytosis, which induces injury in non-infected cells. These $O_2•^-$-mediated pathways contribute to a portion of the extensive tissue injury observed during severe influenza-associated complications [56].

To protect themselves against free radical-mediated damage, cells have developed an antioxidant defense that includes enzymatic and non-enzymatic mechanisms. Free radical generation and a functionally efficient antioxidant defense system must be in equilibrium to avoid cellular damage caused by radicals and their derivatives. Enzymes involved in the elimination of free radicals

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include the superoxide dismutases (SOD), catalase (CAT) and glutathione peroxidase (GPx). In addition to the enzymatic antioxidant system, organisms possess non-enzymatic free radical scavengers, which directly remove toxic reactants because of their electron donating ability. The best known non-enzymatic antioxidants are vitamin E (α-tocopherol), vitamin C (ascorbate), glutathione (GSH), β-carotene and, as recently described, melatonin [25]. Several radical scavengers have been efficacious in ameliorating the severity of viral diseases. N-acetylcysteine, a GSH precursor, inhibits HIV in vitro [89] as did the natural thiol antioxidant, alpha-lipoic acid [90]. Glutathione administration to HIV seropositive individuals by aerosol treatment can correct the glutathione deficiency [91]. The combination of several antioxidants with antiviral drugs synergistically reduces the lethal effects of influenza virus infections [92]. Thus, any agent that functions as a direct radical scavenger and also stimulates antioxidative enzymes could have utility in the treatment of patients with severe complications of viral infections.

Melatonin is a powerful and effective •OH scavenger, which provides protection against oxidative damage of cell components. It also scavenges the peroxyl radical to a lesser degree generated during lipid peroxidation with an activity that, in some situations, is reportedly greater than that of vitamin E [22,93–96]. Also, melatonin directly detoxifies the $ONOO^-$ and possibly peroxynitrous acid (ONOOH) [97]. In vivo, melatonin stimulates several antioxidative enzymes including GPx, CAT and SOD, thereby potentiating its antioxidant properties [98–101]. Melatonin can cross anatomical barriers, including the placenta and the blood–brain barrier [102,103], and easily enter cells [104].

Splenocytes infected with VEEV generated less of NO•, when treated with melatonin; this finding suggests that the indoleamine protected mice infected with the VEEV by a mechanism involving a reduction in NO• concentrations in tissue [105]. Elevated production of NO• and lipid peroxidation products were also found in supernatants and cellular elements of VEEV-infected neuroblastoma cell cultures. Both NO• and lipid peroxidation were decreased by melatonin treatment in a time-dependent manner with an associated reduction in iNOS expression [106]. Production of brain and serum nitrite, as well as neural lipid peroxidation products, was increased in VEEV-infected mice. Melatonin treatment curtailed nitrite concentrations in the brain and serum of infected mice and lowered lipid peroxidation products [107].

Respiratory syncytial virus is a common cause of bronchiolitis, a severe lower respiratory tract affliction that infects nearly all infants by age three worldwide. Mice inoculated intranasal with RSV showed elevated oxidative stress due to rises in NO• and •OH. Also elevated malondialdehyde (MDA) and decreases in GSH and SOD activities were observed. Pre-administration of melatonin in vivo resulted in marked reduction of acute lung oxidative injury induced by RSV, suppressed MDA, NO• and •OH generation, and restored GSH and SOD levels in the lungs of RSV-infected mice [31].

Rabbit hemorrhagic disease virus (RHDV) causes bleeding in the respiratory system, liver, spleen, cardiac muscle,and occasionally in the kidneys of infected rabbits with mortality over 90% in adults [108]. The activity and mRNA expression of the antioxidants enzymes GPx, glutathione-s-transferase (GST) and Mn-SOD were significantly reduced in the liver of RHDV-infected rabbits used as a model of fulminant hepatic failure; these changes were reduced by melatonin administration in a concentration-dependent manner. Melatonin treatment also caused a rise in protein expression of the nuclear factor erythroid 2 (Nrf2), a transcription factor that plays a critical role by binding to the antioxidant response element in the promoter region of a number of genes encoding for antioxidant and detoxifying enzymes in several types of cells and tissues [109]. The activation of Nrf2 during prevention of oxidative liver injury by melatonin in rats treated with dimethylnitrosamine has been reported [110].

MELATONIN AS A POSITIVE REGULATOR OF IMMUNE FUNCTIONS IN VIRAL INFECTIONS

During the early phase of infection and depending on the nature of the infected cells and the infecting virus, early innate defense mechanisms may be triggered to limit the extent of viral spread. The first mechanism to limit the extent of viral spread is the recognition of pathogen-associated molecular patterns (PAMPs), which are mostly viral nucleic acids, or their synthetic analogs produced during the viral infection, by a large repertoire of pattern recognition receptors

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(PRRs), including Toll-like receptors (TLRs), Nod-like receptors (NLRs), RIG-I-like receptors (RLRs) and AIM2-like receptors (ALRs) [111–114]. Such recognition initiates signaling cascades that culminate in the activation of transcription factors including nuclear factor kappa B (NF-κB), activating transcription factor 2 (ATF-2), activating protein-1 (AP-1) and interferon regulatory factors 3 (IRF3) and 7 (IRF7). These stimulate the expression of type I IFN genes that are synthesized in most cell types and especially in plasmacytoid dendritic cells (pDC) [115]. All IFNs bind to specific ubiquitously expressed cell surface receptors and induce a large number of interferon-stimulated genes (ISG), whose encoded proteins mediate the antiviral effects of interferons.

Among these ISGs, dsRNA-activated protein kinase (PKR) primarily inhibits replication of RNA viruses such as vesicular stomatitis virus (VSV), EMCV, WNV, HCV and DNA viruses including HSV-1 [116]. Another group of ISGs is the 20–50-oligoadenylate synthetases (OAS) that requires dsRNA for its activation and is a major antiviral effector against picornaviruses (e.g. EMCV) and influenza A virus, as well as other RNA viruses [117]. Non-specific ssRNA cleavage also occurs after induction of ISG20, a 30-exoribonuclease, which contributes to inhibition of RNA viruses such as VSV [118]. An additional, non-enzymatic mechanism of translation inhibition is pursued by the ISG56/IFIT family proteins, which act against HCV [119–121]. Another IFN-induced protein is the human MxA, which is a key component in innate defense against orthomyxoviruses such as influenza virus as well as measles virus, VSV, Hanta virus and SFV [116,122], the Viperin (CIG5), which might interfere with viral budding of enveloped viruses, such as CMV, HCV, and influenza virus [123], and the nucleic acid-editing enzymes APOBEC3G and −3 F, which inhibit retroviruses [124].

A second mechanism is the triggering of effector functions of cellular components of the innate immune system, such as granulocytes, natural killer cells (NK) and natural killer T cells (NKT cells), macrophages, and dendritic cells, which are normally rapidly recruited and/or activated at the site of virus infection, causing a local inflammation [125]. During this early phase, activated NK cells release IFN-γ, which is not stimulated by viral PAMPs but by IL-12 and IL-18 released by activated macrophages [126]. All of the cellular components of the innate immune system can participate in the antiviral response by killing infected cells, by producing chemokines (including eotaxin, RANTES, MCP-1, IL-8) that recruit inflammatory cells into the infected tissue and by producing antiviral and immunoregulatory cytokines (including TNF-α, IL-1, IL-3, IL-4, IL-5, IL-6, IL-12, IL-18, GM-CSF) that enable the adaptive immune response to recognize infected cells and perform antiviral effector functions [127–130]. Lymphocytes are cells of this adaptive immune system. Among them, two subsets of $CD4^+$ T cells, Th1 and Th2, play a key role in antiviral immunity. After being stimulated by antigen presenting cells, Th1 cells produce IL-2, TNF-α and IFN-γ, which possess antiviral activities and regulate activation of $CD8^+$ cytotoxic T cells, whereas Th2 cells produce IL-4, IL-5, IL-10 and IL-13, which stimulate B cells to produce antibodies [131]. Despite the fact that virus-specific Th2 cells can be detected following primary infection by any virus, virus-specific Th1 cells are usually much more abundant and reach very high numbers at the peak of the acute infection [132]. Moreover, their frequencies remain elevated following resolution of the infection.

Melatonin is synthesized in lymphoid organs, such as the bone marrow, thymus and lymphocytes [133–135], and there are high affinity membrane melatonin receptors as well as nuclear binding sites in circulating lymphocytes, spleen cells and thymocytes [136–138]. Melatonin is known to activate both innate and adaptive immune responses leading to an increase in immune responsiveness and regulation of several immune functions [27,28,139–143]. Melatonin has properties as an inflammatory regulator, because it differentially modulates pro-inflammatory enzymes, and controls the production of inflammatory mediators such as cytokines and leukotrienes. The timing of its pro-inflammatory and anti-inflammatory effects suggests that melatonin might promote early phases of inflammation, on the one hand, and contribute to its attenuation on the other hand, to avoid complications of chronic inflammation [144]. Melatonin enhances the production of IL-1, IL-6, TNF-α and IL-12 from the monocytes [145] and of IL-2, IFN-γ and IL-6 from cultured human peripheral blood mononuclear cells [137]. It has been suggested that melatonin and IFN-γ create an immunoregulatory circuit responsible for the antiviral, antiproliferative and immunomodulatory

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actions of IFN-γ [146]. This cytokine increases serotonin and melatonin levels in lymphocytes and macrophages. The early stimulation in the production of IFN-γ by melatonin suggests that earlier treatment with this indoleamine could increase the antiviral activity of IFN-γ [147]. In addition to stimulating the production of several cytokines that regulate immune function, melatonin enhances immune function by directly stimulating polymorphonuclear cells, macrophages, NK cells and lymphocytes [148]. Recently, considerable attention has been focused on the fact that melatonin treatment has been found to augment CD4+ T cells in lymph nodes of rats [149]. Consequently, melatonin is considered an immunoenhancing agent [141,150].

In retrovirus-infected people and mice, whereas Th1 cytokine (IL-2 and IFN-γ) production declines, Th2 cytokine (IL-4, IL-5, IL-6, and IL-10) production increases [151–153]. The excessive Th2 cytokines suppress Th1 cells, causing anergy of cell-mediated immunity, thus allowing the retrovirus as well as normal flora to reproduce and promote free radical generation by macrophages [154]. Female C57BL/6 mice infected with the LP-BM5 MLV develop murine AIDS. Treatment with melatonin, alone or with dehydroepiandrosterone (DHEA), prevented retrovirus-induced reduction in B-cell and T-cell proliferation and in Th1 cytokine secretion, as well as overproduction of Th2 cytokines and TNF-α [155]. In fact, melatonin alters the balance of Th1 and Th2 cells mainly towards Th1 responses increasing the production of Th1 cytokines [156].

A link between melatonin and the immune system has been also reported in patients infected with HIV-1. Although mean serum IL-12 levels in HIV-1-affected individuals did not significantly differ from healthy controls, the IL-12 levels of HIV-1 patients with advanced disease (CDC stage C) were significantly lower than those of patients in less advanced CDC stages B and A. Taking into account that serum IL-12 levels run parallel with serum melatonin concentrations as the disease advances, a relationship between immune function and melatonin has been suggested; a reduction in serum melatonin could possibly affect IL-12 production thereby contributing to the progress of HIV-1 infection [157].

The protective effect of melatonin against VEEV by regulation of the immune system has been described by Bonilla *et al*. [158]. The endogenous production of IFN-γ, IL-1β and TNF-α, but not of IL-2 and IL-4, is stimulated in VEEV-infected mice treated with melatonin [159]. Nevertheless, the average mortality obtained during neutralization experiments with the corresponding anticytokine antibody suggests that although neither TNF-α nor IFN-γ is essential for the protective effect of melatonin observed in murine VEEV infection, IL-1β induced by melatonin treatment is a target cytokine to promote the immune enhanced state. This in turn causes the viral clearance or helps generate an earlier immune response against the VEEV infection [160]. In contrast, in the brain of VEEV-infected mice, melatonin stimulates the endogenous production of IL-1β but reduces the concentration of TNF-α [158]. IL-1β is considered one of the earliest host mediators during infectious diseases of the CNS and its role in infectious processes of the brain parallels its role in the peripheral immune system [160]. Although IL-1β deficiency is protective against fatal Sindbis virus infection [161], mice deficient in IL-1β have increased susceptibility to influenza virus [162]. In poxvirus animal models, the viral induction of this cytokine is also beneficial for the host [163]. The increase in IL-1β levels detected in blood and in brain of VEEV-infected mice after melatonin treatment also plays a protective role, possibly by neuronal support and protection by inducing nerve growth factor secretion by astrocytes [164]. This supplies a trophic factor for many neuronal cell types in times of stress such as that produced by VEEV infection.

The significant reduction in the concentration of brain TNF-α induced by melatonin in VEEV-infected mice likely diminishes the inflammatory response caused by the migration of granulocytes and macrophages to inflammatory sites within the CNS [165]. These cells are recruited by colony-stimulating factors produced by astrocytes stimulated by TNF-α and as a consequence of alterations in blood–brain barrier (BBB) permeability caused by the adhesive properties of astrocytes stimulated by TNF-α. TNF-α is known to induce intercellular adhesion molecules on neighboring endothelial cells [166], alter BBB permeability and promote inflammatory cell infiltration into the CNS. By reducing adhesion molecule production, which melatonin is known to do [167], the indole would protect the brain infected with VEEV.

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Respiratory syncytial virus bronchiolitis in infants is characterized by a massive infiltration of inflammatory cells into the airways. Of the diverse intracellular signaling pathways, RSV is recognized by TLR3, which initiates a signaling cascade that culminates in the activation of the transcription factor NF-κB; NF-κB is a central mediator of RSV-induced airway inflammation in vivo [145,168,169]. RSV infection of RAW264.7 macrophages time-dependently stimulates the rapid activation of TLR3 and NF-κB, as well as subsequent NF-κB dependent genes, many of which encode for pro-inflammatory cytokines and chemokines including TNF-α and IL-1β. Melatonin decreases TLR3-mediated downstream gene expression in RSV-infected macrophages in a dose-dependent and time-dependent manner. Such inhibition of NF-κB activity, as well as of TNF-α in serum, seems to be the key event required to explain the reduction in inflammatory gene expression caused by melatonin [31,170].

MELATONIN AS A REGULATOR OF PROGRAMMED CELL DEATH IN VIRAL INFECTIONS

As obligate intracellular parasites, viruses are dependent on the host for each stage of replication and, therefore, constantly interface with multiple components of the host cell machinery, including cellular receptors and uptake pathways, gene expression mechanisms and the cell division apparatus. Viral utilization of these systems likely causes cell stress and activates death-signaling pathways or alters expression of genes that control cell survival, evoking PCD [171,172].

Apoptosis is one type of PCD, which is dependent on cleavage of important cellular factors by effector caspases such as caspase-3 and caspase-7. Two major pathways govern the activation of such effector caspases. In the intrinsic pathway, intracellular stresses sensed by the BH3-only members of the bcl-2 family promote the formation of the apoptosome by activation of caspase-9 through release of proapoptotic molecules such as cytochrome c and Smac/Diablo from the mitochondria. The apoptosome directly activates effector caspases. In the extrinsic pathway, occupation of death receptors such as Fas and tumor necrosis factor receptor (TNF-R) by death ligands including FasL and TNFα forms a death-inducing signaling complex (DISC). This results in the activation of the initiator caspase, caspase-8, which directly mediates effector caspase activation and causes cell death.

The ability of melatonin to modulate apoptosis and to differentially regulate the expression of pro-apoptotic and anti-apoptotic mediators has been reported in many studies [29,173–177]. RHDV infection induces liver apoptosis with increased caspase-3 expression and activity [178,179]. These effects are attenuated by melatonin in a concentration-dependent manner. Anti-apoptotic actions of melatonin on the intrinsic pathway were related to a reduced expression of Bax and cytosolic cytochrome c release, increased expression of Bcl-2 and Bcl-xL, and inhibition of caspase-9 activity. Melatonin treatment also has effects on extrinsic pathway resulting in a reduction in caspase-8 activity, TNF-R1 expression and phosphorylated Janus kinase (JNK) expression, and increased expression of cellular FLICE-inhibitory protein (c-FLIP), an inhibitor of caspase-8 [179]. These findings show that inhibition of apoptotic mechanisms contributes to the beneficial effects of melatonin in rabbits with experimental infection by RHDV and supports a potential hepatoprotective role of melatonin in fulminated hepatic failure.

Autophagy is a type of PCD characterized by the formation of autophagosomes to remove excessive proteins and thereby maintains homeostasis within the cell. Autophagy is now recognized as a component of both innate and adaptive immune responses to bacterial and viral pathogens [180]. Varicella zoster virus infection provides an excellent example of autophagy in humans, because abundant autophagosomes are easily detected in the skin vesicles of both varicella and zoster [181]. Autophagy is also found during viral replication of HCV [182], rabbit calicivirus [183] and poliovirus [184]. Given that melatonin modulates autophagy through redox-sensitive transcription factors [185], the role of melatonin in such viral infections involving autophagy should be examined.

MELATONIN AS A CO-TREATMENT IN VIRAL INFECTIONS

Beneficial effects of melatonin when combined with several drugs, such as doxorubicin, cisplatin, epirubicin, cytarabine, bleomycin, gentamicin, cyclosporin, indometacin, acetylsalicylic acid, ranitidine, omeprazole, isoniazid, iron and erythropoietin, phenobarbital, carbamazepine, haloperidol,

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caposide-50, morphine, cyclophosphamide and L-cysteine have been reported [24]. Recently, a single blind randomized study showed a higher percent of a complete regression of symptoms of HSV-1 infection after a treatment with melatonin plus SB-73 (an extract of *Aspergillus* sp. with antiherpetic properties) compared with the treatment with acyclovir alone [186]. Effects of melatonin to increase the efficacy of other antivirals should be studied.

CONCLUDING REMARKS AND PERSPECTIVES

Melatonin is an endogenously produced and ubiquitously acting molecule [187–189]. Because of its highly diverse actions, this indoleamine has potential to combat a wide variety of pathophysiological conditions [190–194]; it has been tested in numerous clinical trials [23] with the outcomes of the treatments always being beneficial. Because of its essential and basic actions on cell physiology, melatonin qualifies for the moniker "molecular handyman," as indicated in the title of this review.

In relation to viral infections, melatonin also seems to be beneficial as indicated in the experimental studies summarized herein. Its favorable actions against viral infections likely relate to its ability to limit the negative molecular processes normally activated when viruses invade cells. Melatonin's actions include an ability to promote immune surveillance, to scavenge free radicals thereby significantly reducing the associated molecular destruction and to modulate the processes related to apoptosis. These multiple actions suggest that melatonin should be evaluated in randomized controlled trials as a preventive agent or as a treatment of viral infections particularly in older individuals where endogenous levels of melatonin have declined. It is the hope of the authors that this summary will stimulate interest in experimental examination of melatonin's antiviral actions.

CONFLICT OF INTEREST

The authors have no competing interest.

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REFERENCES

1. Lerner AB, Case JD, Takahashi Y, Lee TH, Mori N. Isolation of melatonin, a pineal factor that lightens melanocytes. *Journal of the American Chemical Society* 1958; **80**: 2587.
2. Lerner AB, Case JD, Heinzelmann RV. Structure of melatonin. *Journal of the American Chemical Society* 1959; **81**: 6084–6085.
3. Tamarkin L, Baird CJ, Alameida OF. Melatonin: a coordinating signal for mammalian reproduction. *Science* 1985; **227**: 714–720.
4. Armstrong SM, Cassone VM, Chesworth MJ, Redman JR, Short RV. Synchronization of mammalian circadian rhythms by melatonin. *Journal of Neural Transmission. Supplementum* 1986; **21**: 375–394.
5. Reiter RJ. The melatonin rhythm: both a clock and a calendar. *Experientia* 1993; **49**: 654–664.
6. Reiter RJ, Tan DX, Manchester LC, Paredes SD, Mayo JC, Sainz RM. Melatonin and reproduction revisited. *Biology of Reproduction* 2009; **81**: 445–456.
7. Reiter RJ, Tan DX, Sanchez-Barcelo E, Mediavilla MD, Gitto E, Korkmaz A. Circadian mechanisms in the regulation of melatonin synthesis: disruption with light at night and pathophysiological consequences. *Journal of Experimental and Integrative Medicine* 2011; **1**: 13–22.
8. Reiter RJ. Melatonin: that ubiquitously acting pineal hormone. *News in Physiology Science* 1991; **6**: 223–227.
9. Jou MJ, Peng TI, Yu PZ, *et al*. Melatonin protects against common deletion of mitochondrial DNA-augmented mitochondrial oxidative stress and apoptosis. *Journal of Pineal Research* 2007, **43**:389–404.
10. Hardeland R, Poeggeler B. Melatonin beyond its classical functions. *Open Physiology Journal* 2008; **1**: 1–23.
11. Hardeland R, Coto-Montes A. New vistas on oxidative damage and aging. *Open Biology Journal* 2010; **3**: 39–52.
12. Acuna-Castroviejo D, Lopez LC, Escames G, Lopez A, Garcia JA, Reiter RJ. Melatonin-mitochondrial interplay in health and disease. *Current Topics in Medicinal Chemistry* 2011; **11**: 221–240.
13. Jou MJ, Peng TI, Hsu LF, *et al*. Visualization of melatonin's multiple mitochondrial levels of protection against mitochondrial Ca^{2+}-mediated permeability transition and beyond in rat brain astrocytes. *Journal of Pineal Research* 2010; **48**: 20–38.
14. Reiter RJ, Richardson BA, Johnson LY, Ferguson BN, Dinh DT. Pineal melatonin rhythm: reduction in aging Syrian hamsters. *Science* 1980; **210**: 1272–1273.
15. Reiter RJ, Craft CM, Johnson JE Jr, *et al*. Age-associated reduction in nocturnal pineal melatonin levels in female rats. *Endocrinology* 1981; **109**: 1295–1297.
16. Sack RL, Lewy AJ, Erb DL, Vollmer WM, Singer CM. Human melatonin production decreases with age. *Journal of Pineal Research* 1986; **3**: 379–388.
17. de Castro Silva C, de Bruin VMS, Cunho GMA, Nunes DM, Medieros CAM, de Bruin PFC. Melatonin improves sleep and reduces nitrite in the exhaled breath condensate in cystic fibrosis—randomized, double-blind, placebo-controlled study. *Journal of Pineal Research* 2010; **48**: 65–71.
18. Rodella LF, Fillipini F, Bonomini F, Bresciani R, Reiter RJ, Rezzani R.

Beneficial effects of melatonin on nicotine-induced vasculopathy. *Journal of Pineal Research* 2010; **48**: 126–132.

19. Park SY, Jang WJ, Yi EY, *et al*. Melatonin suppresses tumor angiogenesis by inhibiting HIF-1α stabilization under hypoxia. *Journal of Pineal Research* 2010; **48**: 178–184.
20. Chen CF, Wang D, Reiter RJ, Yeh DY. Oral melatonin attenuates lung inflammation and airway hyperactivity induced by aerosolized pancreatic fluid in rats. *Journal of Pineal Research* 2011; **50**: 46–53.
21. Gitto E, Aversa S, Reiter RJ, Barberi I, Pellegrino S. Update on the use of melatonin in pediatrics. *Journal of Pineal Research* 2011; **50**: 21–28.
22. Bonnefont-Rousselot D, Collin F. Melatonin: action as antioxidant and potential applications in human disease and aging. *Toxicology* 2010; **278**: 55–67.
23. Sánchez-Barceló EJ, Mediavilla MD, Tan DX, Reiter RJ. Clinical uses of melatonin: evaluation of human trials. *Current Medicinal Chemistry* 2010; **17**: 2070–2095.
24. Reiter RJ, Tan DX, Sainz RM, Mayo JC, Lopez-Burillo S. Melatonin: reducing the toxicity and increasing the efficacy of drugs. *Journal of Pharmacy and Pharmacology* 2002; **54**: 1299–1321.
25. Tan DX, Chen LD, Poeggeler B, Manchester LC, Reiter RJ. Melatonin: a potent, endogenous hydroxyl radical scavenger. *Endocrine Journal* 1993; **1**: 57–60.
26. Reiter RJ, Tan DX, Manchester LC, Qi W. Biochemical reactivity of melatonin with reactive oxygen and nitrogen species: a review of the evidence. *Cell Biochemistry and Biophysics* 2001; **34**: 237–256.
27. Guerrero JM, Pozo D, García-Mauriño S, Osuna C, Molinero P, Calvo JR. Involvement of nuclear receptors in the enhanced IL-2 production by melatonin in Jurkat cells. *Annals of the New York Academy of Sciences* 2000; **917**: 397–403.
28. Guerrero JM, Reiter RJ, Melatonin-immune system relationships. *Current Topics in Medicinal Chemistry* 2002; **2**: 167–179.
29. Mediavilla MD, Sanchez-Barcelo EJ, Tan DX, Manchester L, Reiter RJ. Basic mechanisms involved in the anti-cancer effects of melatonin. *Current Medicinal Chemistry* 2010; **17**: 4462–4481.
30. Maestroni GJ. Therapeutic potential of melatonin in immunodeficiency states, viral diseases, and cancer. *Advances in Experimental Medicine and Biology* 1999; **467**: 217–226.
31. Huang SH, Cao XJ, Liu W, Shi XY, Wei W. Inhibitory effect of melatonin on lung oxidative stress induced by respiratory syncytial virus infection in mice. *Journal of Pineal Research* 2010; **48**: 109–116.
32. Maestroni GJ, Conti A, Pierpaoli W. Pineal melatonin, its fundamental immunoregulatory role in aging and cancer. *Annals of the New York Academy of Sciences* 1988; **521**: 140–148.
33. Ben-Nathan D, Maestroni GJM, Lustig S, Conti A. Protective effects of melatonin in mice infected with encephalitis viruses. *Archives of Virology* 1995; **140**: 223–230.
34. Bonilla E, Valero-Fuenmayor N, Pons H, Chacín-Bonilla L. Melatonin protects mice infected with Venezuelan equine encephalomyelitis virus. *Cellular and Molecular Life Sciences* 1997; **53**: 430–434.
35. Ellis LC. Melatonin reduces mortality from Aleutian disease in mink (*Mustela vison*). *Journal of Pineal Research* 1996; **21**: 214–217.
36. de Groot H. Reactive oxygen species in tissue injury. *Hepato-Gastroenterology* 1994; **41**: 328–332.
37. Toyokuni S. Reactive oxygen species-induced molecular damage and its application in pathology. *Pathology International* 1999; **49**: 91–102.
38. Granger DL, Hibbs JB Jr, Perfect JR, Durack DT. Specific amino acid (L-arginine) requirement for microbiostatic activity of murine macrophages. *The Journal of Clinical Investigation* 1988; **81**: 1129–1136.
39. Nathan CF, Hibbs JB Jr. Role of nitric oxide synthesis in macrophage antimicrobial activity. *Current Opinion in Immunology* 1991; **3**: 65–70.
40. James SL. Role of nitric oxide in parasitic infections. *Microbiological Reviews* 1995; **59**: 533–547.
41. Nathan C, Shiloh MU. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. *The Journal of Clinical Investigation* 2000; **97**: 8841–8848.
42. Peterhans E, Grob M, Bürge T, Zanoni R. Virus-induced formation of reactive oxygen intermediates in phagocytic cells. *Free Radical Research Communications* 1987; **3**: 39–46.
43. Oda T, Akaike T, Hamamoto T, Suzuki F, Hirano T, Maeda H. Oxygen radicals in influenza-induced pathogenesis and treatment with pyran polymer-conjugated SOD. *Science* 1989; **244**: 974–976.
44. Akaike T, Ando M, Oda T, *et al*. Dependence on O_2 generation by xanthine oxidase of pathogenesis of influenza virus infection in mice. *The Journal of Clinical Investigation* 1990; **85**: 739–745.
45. Ikeda T, Shimokata K, Daikoku T, Fukatsu T, Tsutsui Y, Nishiyama Y. Pathogenesis of cytomegalovirus-associated pneumonitis in ICR mice: possible involvement of superoxide radicals. *Archives of Virology* 1992; **127**: 11–24.
46. Schwartz KB. Oxidative stress during viral infection: a review. *Free Radical Biology & Medicine* 1996; **21**: 641–649.
47. Stuehr DJ, Griffith OW. Mammalian nitric oxide synthase. *Advances in Enzymology and Related Areas of Molecular Biology* 1992; **65**: 287–346.
48. Bukrinsky MI, Nottet HS, Schmidtmayerova H, *et al*. Regulation of nitric oxide synthase activity in human immunodeficiency virus type 1 (HIV-1)-infected monocytes: implications for HIV-associated neurological disease. *The Journal of Experimental Medicine* 1995; **181**: 735–745.
49. Majano PL, García-Monzón C, López-Cabrera M, *et al*. Inducible nitric oxide synthase expression in chronic viral hepatitis. Evidence for a virus-induced gene upregulation. *The Journal of Clinical Investigation* 1998; **101**: 1343–1352.
50. Dustin LB, Rice CM. Flying under the radar: the immunobiology of hepatitis C. *Annual Review of Immunology* 2007; **25**: 71–99.
51. Zaki Mel S, Saudy N, El Diasty A. Study of nitric oxide in patients with chronic hepatitis C genotype 4: relationship to viremia and response to antiviral therapy. *Immunological Investigations* 2010; **39**: 598–610.
52. Koprowski H, Zheng YM, Heber-Katz E, *et al*. In vivo expression of inducible nitric oxide synthase in experimentally induced neurologic diseases. *Proceedings of the*

National Academy of Science USA 1993; **90**: 3024–7.

53. Zheng YM, Schäfer MK, Weihe E, *et al*. Severity of neurological signs and degree of inflammatory lesions in the brains of the rats with Borna disease correlate with the induction of nitric oxide synthase. *Journal of Virology* 1993; **67**: 5786–5791.
54. Karupiah G, Xie QW, Buller RM, Nathan C, Duarte C, MacMicking JD. Inhibition of viral replication by interferon-gamma-induced nitric oxide synthase. *Science* 1993; **261**: 1445–1448.
55. Akaike T, Noguchi Y, Ijiri S, *et al*. Pathogenesis of influenza virus-induced pneumonia: involvement of both nitric oxide and oxygen radicals. *Proceedings of the National Academy of Science USA* 1996; **93**: 2448–2453.
56. Akaike T, Suga M, Maeda H. Free radicals in viral pathogenesis: molecular mechanisms involving superoxide and NO. *Proceedings of the Society for Experimental Biology and Medicine* 1998; **217**: 64–73.
57. Fujii S, Akaike T, Maeda H. Role of nitric oxide in pathogenesis of herpes simplex virus encephalitis in rats. *Virology* 1999; **256**: 203–212.
58. Akaike T, Maeda H. Pathophysiological effects of high-output production of nitric oxide. In Nitric Oxide: Biology and Pathobiology, Ignarro LJ (ed.). Academic Press: San Diego, CA, 2000; 733–745.
59. Akaike T, Maeda H. Nitric oxide and virus infection. *Immunology* 2000; **101**: 300–308.
60. Cunha FQ, Moncada S, Liew FY. Interleukin-10 (IL-10) inhibits the induction of nitric oxide synthase by interferon-gamma in murine macrophages. *Biochemical and Biophysical Research Communications* 1992; **182**: 1155–1159.
61. Vodovotz Y, Bogdan C, Paik J, Xie QW, Nathan C. Mechanisms of suppression of macrophage nitric oxide release by transforming growth factor beta. *The Journal of Experimental Medicine* 1993; **178**: 605–613.
62. Bogdan C, Vodovotz Y, Paik J, Xie QW, Nathan C. Mechanism of suppression of nitric oxide synthase expression by interleukin-4 in primary mouse macrophages. *Journal of Leukocyte Biology* 1994; **55**: 227–233.
63. Adamson DC, Kopnisky KL, Dawson TM, Dawson VL. Mechanisms and structural determinants of HIV-1coat protein, gp41-induced neurotoxicity. *Journal of Neuroscience* 1999; **19**: 64–71.
64. Hori K, Burd PR, Furuke K, *et al*. Human immunodeficiency virus-1-infected macrophages induce inducible nitric oxide synthase and nitric oxide (NO) production on astrocytes: astrocytic NO as a possible mediator of neuronal damage in acquired immunodeficiency syndrome. *Blood* 1999; **93**: 1843–1850.
65. Tsutsumi H, Takeuchi R, Ohsaki M, Seki K, Chiba S. Respiratory syncytial virus infection of human respiratory epithelial cells enhances inducible nitric oxide synthase gene expression. *Journal of Leukocyte Biology* 1999; **66**: 99–104.
66. Croen KD. Evidence for an antiviral effect of nitric oxide. Inhibition of herpes simplex virus type 1 replication. *The Journal of Clinical Investigation* 1993; **91**: 2446–2452.
67. Mannick JB, Asano K, Izumi K, Kieff E, Stamler JS. Nitric oxide produced by human B lymphocytes inhibits apoptosis and Epstein–Barr virus reactivation. *Cell* 1994; **79**: 1137–1146.
68. Karupiah G, Chen JH, Nathan CF, Mahalingam S, MacMicking JD. Identification of nitric oxide synthase 2 as an innate resistance locus against ectromelia virus infection. *Journal of Virology* 1998; **72**: 7703–7706.
69. Gao X, Tajima M, Sairenji T. Nitric oxide downregulates Epstein–Barr virus reactivation in epithelial cell lines. *Virology* 1999; **258**: 375–381.
70. Saura M, Zaragoza C, McMillan A, *et al*. An antiviral mechanism of nitric oxide: inhibition of a viral proteinase. *Immunity* 1999; **10**: 21–28.
71. Zaragoza C, Ocampo CJ, Saura M, *et al*. Inducible nitric oxide synthase protection against coxsackievirus pancreatitis.*Journal of Immunology* 1999; **163**: 5497–5504.
72. Akaike T, Maeda H. Nitric oxide in influenza. In Nitric Oxide in Infection, Fang FC (ed.). Kluwer Academic/Plenum Publishers: New York, 1999; 397–415.
73. van den Broek M, Bachmann MF, Köhler G, *et al*. IL-4 and IL-10 antagonize IL-12-mediated protection against acute vaccinia virus infection with a limited role of IFN-gamma and nitric oxide synthetase 2. *Journal of Immunology* 2000; **164**: 371–378.
74. Karupiah G, Chen JH, Mahalingam S, Nathan CF, MacMicking JD. Rapid interferon gamma-dependent clearance of influenza A virus and protection from consolidating pneumonitis in nitric oxide synthase 2-deficient mice. *The Journal of Experimental Medicine* 1998; **188**: 1541–1546.
75. Bartholdy C, Nansen A, Christensen JE, Marker O, Thomsen AR. Inducible nitric-oxide synthase plays a minimal role in lymphocytic choriomeningitis virus induced, T cell-mediated protective immunity and immunopathology. *The Journal of General Virology* 1999; **80**: 2997–3005.
76. Wu GF, Pewe L, Perlman S. Coronavirus-induced demyelination occurs in the absence of inducible nitric oxide synthase. *Journal of Virology* 2000; **74**: 7683–7686.
77. Guillemard E, Varano B, Belardelli F, Quero AM, Gessani S. Inhibitory activity of constitutive nitric oxide on the expression of alpha/beta interferon genes in murine peritoneal macrophages. *Journal of Virology* 1999; **73**: 7328–7333.
78. Kreil TR, Eibl MM. Nitric oxide and viral infection: no antiviral activity against a flavivirus in vitro, and evidence for contribution to pathogenesis in experimental infection in vivo. *Virology* 1996; **219**: 304–306.
79. Adler H, Beland JL, Del-Pan NC, *et al*. Suppression of herpes simplex virus type 1 (HSV-1)-induced pneumonia in mice by inhibition of inducible nitric oxide synthase (iNOS, NOS2). *The Journal of Experimental Medicine* 1997; **185**: 1533–1540.
80. Nishio R, Matsumori A, Shioi T, Ishida H, Sasayama S. Treatment of experimental viral myocarditis with interleukin-10. *Circulation* 1999; **100**: 1102–1108.
81. Hirasawa K, Jun HS, Han HS, Zhang ML, Hollenberg MD, Yoon JW. Prevention of encephalomyocarditis virus-induced diabetes in mice by inhibition of the tyrosine kinase signaling pathway and subsequent suppression of nitric oxide production in macrophages. *Journal of Virology* 1999; **73**: 8541–8548.

82. Andrews DM, Matthews VB, Sammels LM, Carrello AC, McMinn PC. The severity of Murray Valley encephalitis in mice is linked to neutrophil infiltration and inducible nitric oxide synthase activity in the central nervous system. *Journal of Virology* 1999; **73**: 8781–8790.
83. Maeda H, Akaike T. Oxygen free radicals as pathogenic molecules in viral diseases. *Proceedings of the Society for Experimental Biology and Medicine* 1991; **198**: 721–727.
84. Sidwell RW, Huffman JH, Bailey KW, Wong MH, Nimrod A, Panet A. Inhibitory effects of recombinant manganese superoxide dismutase on influenza virus infections in mice. *Antimicrobial Agents and Chemotherapy* 1996; **40**: 2626–2631.
85. González-Gallego J, García-Mediavilla MV, Sánchez-Campos S. Hepatitis C virus, oxidative stress and steatosis: current status and perspectives. *Current Molecular Medicine* 2011; **11**: 373–390.
86. Bureau C, Bernad J, Chaouche N, *et al*. Non-structural 3 protein of hepatitis C virus triggers ROS production in human monocytes via activation of NADPH oxidase. *Journal of Biological Chemistry* 2001; **276**: 23077–23083.
87. Hiraku Y, Tabata T, Ma N, Murata M, Ding X, Kawanishi S. Nitrative and oxidative DNA damage in cervical intraepithelial neoplasia associated with human papilloma virus infection. *Cancer Science* 2007; **98**: 964–972.
88. Lassoued S, Ben Ameur R, Ayadi W, Gargouri B, Ben Mansour R, Attia H. Epstein–Barr virus induces an oxidative stress during the early stages of infection in B lymphocytes, epithelial, and lymphoblastoid cell lines. *Molecular and Cellular Biochemistry* 2008; **313**: 179–186.
89. Newman GW, Balcewicz-Sablinska MK, Guarnaccia JR, Remold HG, Silberstein DS. Opposing regulatory effects of thioredoxin and eosinophil cytotoxicity-enhancing factor on the development of human immunodeficiency virus 1. *The Journal of Experimental Medicine* 1994; **180**: 359–363.
90. Suzuki YJ, Aggarwal BB, Packer L. Alpha-lipoic acid is a potent inhibitor of NF-kappa B activation in human T cells. *Biochemical and Biophysical Research Communications* 1992; **189**: 1709–1715.
91. Holroyd KJ, Buhl R, Borok Z, *et al*. Correction of glutathione deficiency in the lower respiratory tract of HIV seropositive individuals by glutathione aerosol treatment. *Thorax* 1993; **48**: 985–989.
92. Uchide N, Toyoda H. Antioxidant therapy as a potential approach to severe influenza-associated complications. *Molecules* 2011; **16**: 2032–2052.
93. Pieri C, Marra M, Moroni F, Recchioni R, Marcheselli F. Melatonin: a peroxyl radical scavenger more effective than vitamin E. *Life Sciences* 1994; **55**: PL271-PL276.
94. Reiter RJ, Melchiorri D, Sewerynek E, *et al*. A review of the evidence supporting melatonin's role as an antioxidant. *Journal of Pineal Research* 1995; **18**: 1–11.
95. Reiter RJ. Functional aspects of the pineal hormone melatonin in combating cell and tissue damage induced by free radicals. *European Journal of Endocrinology* 1996; **134**: 412–420.
96. Galano A, Tan DX, Reiter RJ. Melatonin as a natural ally against oxidative stress: a physiochemical examination. *Journal of Pineal Research* 2011; **51**: 1–16.
97. Hardeland R. Melatonin and its metabolites as anti-nitrosating and anti-nitrating agents. *Journal of Experimental and Integrative Medicine* 2011; **1**: 67–81.
98. Pablos MI, Agapito MT, Gutierrez R, *et al*. Melatonin stimulates the activity of the detoxifying enzyme glutathione in several tissues of chicks. *Journal of Pineal Research* 1995, **19**:111–115.
99. Coto-Montes A, Boga JA, Tomás-Zapico C, *et al*. Physiological oxidative stress model: Syrian hamster Harderian gland-sex differences in antioxidant enzymes. *Free Radical Biology & Medicine* 2001, **30**:785–792.
100. Rodriguez C, Mayo JC, Sainz RM, *et al*. Regulation of antioxidant enzymes: a significant role for melatonin. *Journal of Pineal Research* 2004; **36**: 1–9.
101. Tomas-Zapico C, Coto-Montes A. A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes. *Journal of Pineal Research* 2005; **39**: 99–104.
102. Reiter RJ, Acuña-Castroviejo D, Tan DX, Burkhardt S. Free radical-mediated molecular damage. *Ann NY Academy Sciences* 2001; **939**: 200–215.
103. Lanoix D, Lacasse AA, Reiter RJ, Vaillancourt C. Melatonin, the smart killer. The human trophoblast as a model. *Molecular and Cellular Endocrinology* 2012; **348**: 1–111.
104. Venegas C, Garcia JA, Escames G, *et al*. Extra pineal melatonin: analysis of subcellular distribution and daily fluctuations. *Journal of Pineal Research* 2012; **52**: 217–227.
105. Valero N, Meleán E, Bonilla E, *et al*. In vitro, melatonin treatment decreases nitric oxide levels in murine splenocytes cultured with the Venezuelan equine encephalomyelitis virus. *Neurochemical Research* 2005; **30**: 1439–1442.
106. Valero N, Espina LM, Mosquera J. Melatonin decreases nitric oxide production, inducible nitric oxide synthase expression and lipid peroxidation induced by Venezuelan encephalitis equine virus in neuroblastoma cell cultures. *Neurochemical Research* 2006; **31**: 925–932.
107. Valero N, Espina LM, Bonilla E, Mosquera J. Melatonin decreases nitric oxide production and lipid peroxidation and increases interleukin-1 beta in the brain of mice infected by the Venezuelan equine encephalomyelitis virus. *Journal of Pineal Research* 2007; **42**: 107–112.
108. Parra F, Prieto M. Purification and characterization of a calicivirus as the causative agent of a lethal hemorrhagic disease in rabbits. *Journal of Virology* 1990; **64**: 4013–4015.
109. Crespo I, Miguel BS, Laliena A, *et al*. Melatonin prevents the decreased activity of antioxidant enzymes and activates nuclear erythroid 2-related factor 2 signaling in an animal model of fulminant hepatic failure of viral origin. *Journal of Pineal Research* 2010; **49**: 193–200.
110. Jung KH, Hong SW, Zheng HM, *et al*. Melatonin ameliorates cerulein-induced pancreatitis by the modulation of nuclear erythroid 2-related factor 2 and nuclear factor-kappaB in rats. *Journal of Pineal Research* 2010; **48**: 239–250.
111. Brennan K, Bowie AG. Activation of host pattern recognition receptors by viruses. *Current Opinion in Microbiology* 2010; **13**: 503–507.

112. Kanneganti TD. Central roles of NLRs and inflammasomes in viral infection. *Nature Reviews Immunology* 2010; **10**: 688–698.
113. Mikula I Jr, Pastoreková S, Mikula I Sr. Toll-like receptors in immune response to the viral infections. *Acta Virologica* 2010; **54**: 231–245.
114. Loo YM, Gale M Jr. Immune signaling by RIG-I-like receptors. *Immunity* 2011; **34**: 680–692.
115. Fensteri V, Sen GC. Interferons and viral infections. *BioFactors* 2009; **35**: 14–20.
116. Sadler AJ, Williams BR. Interferon-inducible antiviral effectors. *Nature Reviews Immunology* 2008; **8**: 559–568.
117. Silverman RH. Viral encounters with 20,50-oligoadenylate synthetase and RNase L during the interferon antiviral response. *Journal of Virology* 2007; **81**: 12720–12729.
118. Espert L, Degols G, Gongora C, *et al*. ISG20, a new interferon-induced RNase specific for single-stranded RNA, defines an alternative antiviral pathway against RNA genomic viruses. *Journal of Biological Chemistry* 2003; **278**: 16151–16158.
119. White CL, Sen GC. Interferons and antiviral action. In Cellular Signaling and Innate Immune Responses to RNA Virus Infections. Brasier AR, Garcia-Sastre A, Lemon SM (eds.) ASM Press: Washington, DC, 2002; 91–106.
120. Terenzi F, Hui DJ, Merrick WC, Sen GC. Distinct induction patterns and functions of two closely related interferon-inducible human genes, ISG54 and ISG56. *Journal of Biological Chemistry* 2006; **281**: 34064–34071.
121. Wang C, Pflugheber J, Sumpter R Jr, *et al*. Alpha interferon induces distinct translational control programs to suppress hepatitis C virus RNA replication. *Journal of Virology* 2003; **77**: 3898–3912.
122. Haller O, Kochs G, Weber F. Interferon, Mx, and viral countermeasures. *Cytokine & Growth Factor Reviews* 2007; **18**: 425–433.
123. Randall RE, Goodbourn S. Interferons and viruses: an interplay between induction, signaling, antiviral responses and virus countermeasures. *The Journal of General Virology* 2008; **89**: 1–47.
124. Aguiar RS, Peterlin BM. APOBEC3 proteins and reverse transcription. *Virus Research* 2008; **134**: 74–85.
125. Guidotti LG, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. *Annual Review of Immunology* 2001; **19**: 65–91.
126. Malmgaard L. Induction and regulation of IFNs during viral infections. *Journal of Interferon and Cytokine Research* 2004; **24**: 439–454.
127. Benyon RC, Bissonnette EY, Befus AD. Tumor necrosis factor-alpha dependent cytotoxicity of human skin mast cells is enhanced by anti-IgE antibodies. *Journal of Immunology* 1991; **147**: 2253–2258.
128. Bradding P, Feather IH, Wilson S, *et al*. Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *Journal of Immunology* 1993; **151**: 3853–3865.
129. Kaplan AP, Kuna P, Reddigari SR. Chemokines and the allergic response. *Experimental Dermatology* 1995; **4**: 260–265.
130. Sampson AP. The role of eosinophils and neutrophils in inflammation. *Clinical and Experimental Allergy* 2000; **1**(Suppl.): 22–27.
131. Mosmann TR, Coffman RL. Th-1and Th-2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annual Review of Immunology* 1989; **7**: 145–173.
132. Varga SM, Welsh RM. High frequency of virus-specific interleukin-2- producing CD4(+) T cells and Th1 dominance during lymphocytic choriomeningitis virus infection. *Journal of Virology* 2000; **74**: 4429–4432.
133. Tan DX, Manchester LC, Reiter RJ, *et al*. Identification of highly elevated levels of melatonin in bone marrow: its origin and significance. *Biochimica et Biophysica Acta* 1999; **1471**: 206–214.
134. Kvetnoy IM. Extrapineal melatonin: location and role within diffuse neuroendocrine system. *The Histochemical Journal* 1999; **31**: 1–12.
135. Carrillo-Vico A, Calvo JR, Abreu P, *et al*. Evidence of melatonin synthesis by human lymphocytes and its physiological significance: possible role as intracrine, autocrine, and/or paracrine substance. *The FASEB Journal* 2004; **18**: 537–539.
136. Pozo D, Delgado M, Fernandez-Santos JM, *et al*. Expression of the Mel1a-melatonin receptor mRNA in T and B subsets of lymphocytes from rat thymus and spleen. *The FASEB Journal* 1997; **11**: 466–473.
137. Garcia-Mauriño S, Gonzalez-Haba MG, Calvo IR, *et al*. Melatonin enhances IL-2, lL-6, and IFN-gamma production by human circulating CD4+ cells: a possible nuclear receptor-mediated mechanism involving T helper type I lymphocytes and monocytes. *Journal of Immunology* 1997; **159**: 574–581.
138. Lardone PJ, Carrillo-Vico A, Molinero P, Rubio A, Guerrero JM. A novel interplay between membrane and nuclear melatonin receptors in human lymphocytes: significance in IL-2 production. *Cellular and Molecular Life Sciences* 2009; **66**: 516–525.
139. Carrillo-Vico A, Guerrero JM, Lardone PJ, Reiter RJ. A review of the multiple actions of melatonin on the immune system. *Endocrine* 2005; **27**: 189–200.
140. Lardone PJ, Alvarez-García O, Carrillo-Vico A, *et al*. Inverse correlation between endogenous melatonin levels and oxidative damage in some tissues of SAMP8 mice. *Journal of Pineal Research* 2006; **40**: 153–157.
141. Szczepanik M. Melatonin and its influence on immune system. *Journal of Physiology and Pharmacology* 2007; **58**: 115–124.
142. Caballero B, Vega-Naredo I, Sierra V, *et al*. Autophagy upregulation and loss of NF-kappaB in oxidative stress-related immunodeficient SAMP8 mice. *Mechanisms of Ageing and Development* 2009; **130**: 722–730.
143. Belyaer O, Herzog T, Munding J, *et al*. Protective role of endogenous melatonin in the early course of acute pancreatitis. *Journal of Pineal Research* 2011; **50**: 71–77.
144. Radogna F, Diederich M, Ghibelli L. Melatonin: a pleiotropic molecule regulating inflammation. *Biochemical Pharmacology* 2010; **80**: 1844–1852.
145. Morrey KM, McLachlan JA, Serkin CD, Bakouche O. Activation of human monocytes by the pineal hormone melatonin. *Journal of Immunology* 1994; **153**: 2671–2680.
146. Finocchiaro LM, Arzt ES, Fernandez-Castelo S, Crisculo M, Finkielman S, Nahmod VE.

Serotonin and melatonin synthesis in peripheral blood mononuclear cells: stimulation by interferon-gamma as part of an immunomodulatory pathway. *Journal of Interferon Research* 1988; **8**: 705–716

147. Bonilla E, Nereida V, Chacín-Bonilla L, Medina-Leendertz S. Melatonin and viral infections. *Journal of Pineal Research* 2004; **36**: 73–79.
148. Currier NL, Sun LZ, Miller SC. Exogenous melatonin: quantitative enhancement in vivo of cells mediating non-specific immunity. *Journal of Neuroimmunology* 2000; **104**: 101–108.
149. Castrillon PO, Esquifino AI, Varas A, Zapata A, Cutrera RA, Cardinali DP. Effect of melatonin treatment on 24-h variations in responses to mitogens and lymphocyte subset populations in rat submaxillary lymph nodes. *Journal of Neuroendocrinology* 2000; **12**: 758–765.
150. Carrillo-Vico A, Reiter RJ, Lardone PJ, *et al*. The modulatory role of melatonin on immune responsiveness. *Current Opinion in Investigational Drugs* 2006; **7**: 423–431.
151. Gazzinelli RT, Makino M, Chattopadhyay SK, *et al*. CD4+ subset regulation in viral infection. Preferential activation of Th2 cells during progression of retrovirus-induced immunodeficiency in mice. *Journal of Immunology* 1992; **148**: 182–188.
152. Bradley WG, Ogata N, Good RA, Day NK. Alteration of in vivo cytokine gene expression in mice infected with a molecular clone of the defective MAIDS virus. *Journal of Acquired Immune Deficiency Syndromes* 1994; **7**: 1–9.
153. Clerici M, Hakim FT, Venzon DJ, *et al*. Changes in interleukin-2 and interleukin-4 production in asymptomatic, human immunodeficiency virus-seropositive individuals. *The Journal of Clinical Investigation* 1993; **91**: 759–765.
154. Wang Y, Huang DS, Liang B, Watson RR. Nutritional status and immune responses in mice with murine AIDS are normalized by vitamin E supplementation. *Journal of Nutrition* 1994; **124**: 2024–2032.
155. 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.
156. Srinivasan V, Spence DW, Trakhi I, Pandi-Perumal SR, Cardinali DP, Maestroni GJ. lmmunomodulation by melatonin: its significance for seasonally occurring diseases. *Neuroimmuno-modulation* 2008; **15**: 93–101.
157. Nunnari G, Nigro L, Palermo F, Leto D, Pomerantz RJ, Cacopardo B. Reduction of serum melatonin levels in HIV-1-infected individuals' parallel disease progression: correlation with serum interleukin-12 levels. *Infection* 2003; **31**: 379–382.
158. Bonilla E, Rodón C, Valero N, *et al*. Melatonin prolongs survival of immunodepressed mice infected with the Venezuelan equine encephalomyelitis virus. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2001; **95**: 207–210.
159. Valero N, Bonilla E, Pons H, *et al*. Melatonin induces changes to serum cytokines in mice infected with the Venezuelan equine encephalomyelitis virus. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2002; **96**: 348–351.
160. Dinarello CA. The biological properties of interleukin-1. *European Cytokine Network* 1994; **5**: 517–531.
161. Liang XH, Goldman JE, Jiang HH, Levine B. Resistance of interleukin-1 beta-deficient mice to fatal Sindbis virus encephalitis. *Journal of Virology* 1999; **73**: 2563–2567.
162. Kozak W. Thermal and behavioural effects of lipopolysaccharide and influenza in interleukin-1 beta deficient mice. *American Journal of Physiology* 1995; **269**: R969–R977.
163. 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.
164. Carman-Krzan M, Wise BC. Arachidonic acid lipoxygenation may mediate interleukin-1 stimulation of nerve growth factor secretion in astroglial cultures. *Journal of Neuroscience Research* 1993; **34**: 225–232.
165. Tweardy DJ, Glazer EW, Mott PL, Anderson K. Modulation by tumor necrosis factor-a of human astroglial cell production of granulocyte-macrophage colony stimulating factor (G-CSF).*Journal of Neuroimmunology* 1991; **32**: 269–278.
166. McCarron RM, Wang L, Racke MK, McFarlin DE, Spatz M. Cytokine-regulated adhesion between encephalitogenic T lymphocytes and cerebrovascular endothelial cells. *Journal of Neuroimmunology* 1993; **43**: 23–30.
167. Baydas G, Nedzvetsky NS, Nerush PA, Kiricheuko SV, Demchenko HM, Reiter RJ. A novel role for melatonin: regulation of the expression of cell adhesion molecules in the rat hippocampus and cortex. *Neuroscience Letters* 2002; **326**: 109–112.
168. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-jB by Toll-like receptor 3. *Nature* 2001; **413**: 732–738.
169. Tian B, Zhang Y, Luxon BA, *et al*. Identification of NF-kappaB-dependent gene networks in respiratory syncytial virus-infected cells. *Journal of Virology* 2002; **76**: 6800–6814.
170. Huang SH, Cao XJ, Wei W. Melatonin decreases TLR3-mediated inflammatory factor expression via inhibition of NF-kappa B activation in respiratory syncytial virus-infected RAW264.7 macrophages. *Journal of Pineal Research* 2008; **45**: 93–100.
171. Koyama AH, Irie H, Fukumori T, Hata S, Iida S, *et al*. Role of virus-induced apoptosis in a host defense mechanism against virus infection. *The Journal of Medical Investigation* 1998, **45**:37–45.
172. Danthi P. Enter the kill zone: initiation of death signaling during virus entry. *Virology* 2011; **411**: 316–324.
173. Sainz RM, Mayo JC, Rodriguez C, Tan DX, Lopez-Burillo S, Reiter RJ. Melatonin and cell death: differential actions on apoptosis in normal and cancer cells. *Cellular and Molecular Life Sciences* 2003; **60**: 1407–1426.
174. Casao A, Mendoza N, Perez-Pe R, *et al*. Melatonin prevents capacitation and apoptotic-like changes in ram spermatozoa and increases fertility. *Journal of Pineal Research* 2010; **48**: 39–46.
175. Kim CH, Kim KH, Yoo YM. Melatonin protects against apoptotic and autophagic cell death in C2 C12 murine myoblast cells. *Journal of Pineal Research* 2011; **50**: 241–249.
176. Espino J, Bejarano I, Paredes SD, Barriga C, Rodriguez AB, Pariente JA. Protective effect of melatonin against human

leucocyte apoptosis induced by intracellular calcium overload: relation with its antioxidant actions. *Journal of Pineal Research* 2011; **51**: 195–206.

177. Koh W, Jeong SJ, Lee HJ, *et al*. Melatonin promotes puromycin-induced apoptosis with activation of caspase-3 and 5[1]-adenosine monophosphate-activated kinase-alpha in human leukemia HL-60 cells. *Journal of Pineal Research* 2011; **50**: 369–373.
178. Alonso C, Oviedo JM, Martín-Alonso JM, Díaz E, Boga JA, Parra F. Programmed cell death in the pathogenesis of rabbit hemorrhagic disease. *Archives of Virology* 1998; **143**: 321–332.
179. Tuñón MJ, San Miguel B, Crespo I, *et al*. Melatonin attenuates apoptotic liver damage in fulminant hepatic failure induced by the rabbit hemorrhagic disease virus. *Journal of Pineal Research* 2011; **50**: 38–45.
180. Grose C. Autophagy during common bacterial and viral infections of children. *Pediatric Infectious Disease Journal* 2010; **29**: 1040–1042.
181. Takahashi MN, Jackson W, Laird DT, *et al*. Varicella-zoster virus infection induces autophagy in both cultured cells and human skin vesicles. *Journal of Virology* 2009; **83**: 5466–5476.
182. Dreux M, Chisari FV. Autophagy proteins promote hepatitis C virus replication. *Autophagy* 2009; **5**: 1224–1225.
183. Casais R, Molleda LG, Machín A, *et al*. Structural and functional analysis of virus factories purified from Rabbit vesivirus-infected Vero cells. *Virus Research* 2008; **137**: 112–121.
184. Suhy DA, Giddings TH Jr., Kirkegaard K. Remodeling the endoplasmic reticulum by poliovirus infection and by individual viral proteins: an autophagy-like origin for virus-induced vesicles. *Journal of Virology* 2000; **74**: 8953–8965.
185. Vega-Naredo I, Caballero B, Sierra V, *et al*. Melatonin modulates autophagy through a redox-mediated action in female Syrian hamster Harderian gland controlling cell types and gland activity. *Journal of Pineal Research* 2012; **52**: 80–92.
186. Nunes Oda S, Pereira Rde S. Regression of herpes viral infection symptoms using melatonin and SB-73: comparison with Acyclovir. *Journal of Pineal Research* 2008; **44**: 373–378.
187. Reiter RJ. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. *Endocrine Reviews* 1991; **12**: 151–180.
188. Reiter RJ, Tan DX, Fuentes-Broto L. Melatonin: a multitasking molecule. *Progress in Brain Research* 2010; **181**: 127–151.
189. Stehle J, Saade A, Rawashdeh O, *et al*. A survey of molecular details in the human pineal gland in the light of phylogeny, structure, function and chronobiological diseases. *Journal of Pineal Research* 2011; **51**: 17–43.
190. Jung-Hynes B, Reiter RJ, Ahmad N. Sirtuins, melatonin and circadian rhythms: building a bridge between aging and cancer. *Journal of Pineal Research* 2010; **48**: 9–19.
191. Paradies G, Petroillo G, Paradies V, Reiter RJ, Ruggiero FM. Melatonin, cardiolipin and mitochondrial bioenergetics in health and disease. *Journal of Pineal Research* 2010; **48**: 297–310.
192. Rosenstein RE, Pandi-Perumal SR, Srinivasan V, Spence DW, Brown GM, Cardinali DP. Melatonin as a therapeutic tool in ophthalmology: implications for glaucoma and uveitis. *Journal of Pineal Research* 2010; **49**: 1–13.
193. Reiter RJ, Tan DX, Korkmaz A, Fuentes-Broto C. Drug-mediated ototoxicity and tinnitus: alleviation with melatonin. *Journal of Physiology and Pharmacology* 2011; **62**: 151–154.
194. Swarnakar S, Paul S, Singh LP, Reiter RJ. Matrix metalloproteinases in health and disease: regulation by melatonin. *Journal of Pineal Research* 2011; **50**: 8–20.