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A Review of the Multiple Actions of Melatonin on the Immune System

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This review summarizes the numerous observations published in recent years which have shown that one of the most significant of melatonin's pleiotropic effects is the regulation of the immune system. The overview summarizes the immune effects of pinealectomy and the association between rhythmic melatonin production and adjustments in the immune system as markers of melatonin's immunomodulatory actions. The effects of both in vivo and in vitro melatonin administration on non-specific, humoral, and cellular immune responses as well as on cellular proliferation and immune mediator production are presented. One of the main features that distinguishes melatonin from the classical hormones is its synthesis by a number of non-endocrine extrapineal organs, including the immune system. Herein, we summarize the presence of immune system–synthesized melatonin, its direct immunomodulatory effects on cytokine production, and its masking effects on exogenous melatonin action. The mechanisms of action of melatonin in the immune system are also discussed, focusing attention on the presence of membrane and nuclear receptors and the characterization of several physiological roles mediated by some receptor analogs in immune cells. The review focuses on melatonin's actions in several immune pathologies including infection, inflammation, and autoimmunity together with the relation between melatonin, immunity, and cancer.

Key Words: Melatonin; immune system; pineal gland; neuroimmunomodulation; extrapineal melatonin; melatonin receptors; immune pathologies.

Introduction

Melatonin (*N*-acetyl-5-methoxy-triptamine) was first isolated in 1958 from the bovine pineal gland by Lerner et al. *(1)*. During the 1960s, the essential keys of the melatonin metabolic pathway were revealed, by which tryptophan (Trp), taken up from the bloodstream, is metabolized to melatonin through four successive well-defined intracellular steps enzymatically catalyzed by tryptophan hydroxylase (EC 1.14.16.4, TPH), aromatic amino acid decarboxylase (EC 4.1.1.28, AAAD), arylalkylamine-*N*-acetyltransferase (EC 2.3.1.87; AA-NAT), and hydroxyindole-*O*-methyltransferase (EC 2.1.1.4; HIOMT), respectively *(2–4)*.

The reciprocal relationship between the pineal gland and the suprachiasmatic nucleus (SCN), the central circadian pacemaker, is a major mechanism of melatonin production. In mammals, melatonin functions as the biological signal of darkness, because the duration of its release from the pineal gland is proportional to night length. Consequently, this temporal information is used to inform other systems of the time of the year and time of day. The chronobiotic properties of melatonin have been established as an important physiological function of the indoleamine *(5)* and one of the reasons for its clinical relevance in functional processes related to period and phase shifts such as jet lag and shiftwork *(6)*. Additionally, melatonin shows a remarkable functional versatility by exhibiting antioxidant, oncostatic, anti-aging, and immunomodulatory properties, among others *(7,8)*. Currently, melatonin has been detected in many organs and in all organisms when appropriate extraction and detection methods are applied. Thus, its remarkable functional versatility is reflected in its wide distribution within phylogenetically distant organisms from bacteria to human beings. Melatonin is also present in plants, vegetables, fruits, seeds, rice, wheat, and medicinal herbs *(9)*.

When melatonin was first isolated, it was considered as an exclusive hormone to the pineal gland; however, over the last few years, the development of highly specific antibodies and detection methods has allowed the identification of melatonin in a large number of non-endocrine extrapineal sites *(10,11)*, including the immune system *(12)*.

Melatonin's molecular mechanisms involve several actions, i.e., via high-affinity G protein–coupled membrane receptors, interaction with cytosol and nuclear proteins, and both direct radical scavenging and redox-modulated processes *(13)*.

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In this review, we summarize the wide range of actions of melatonin, a ubiquitous compound with pleiotropic effects both of an endocrine and a non-endocrine nature in the immune system.

Pineal Melatonin and the Immune System: Endocrine Actions

The relationship between nervous, endocrine, and immune systems is one of the most noteworthy discoveries in modern biology; these systems use a common chemical language for intra- and intersystem communication *(14)*. In this framework, currently, pineal-synthesized melatonin is considered one of members of the complex neuro–endocrine–immunological system.

The first evidence that indicated a possible interrelation between the pineal gland and immune system was proposed in 1926 by Berman who fed kittens for 2 yr with pineal glands from young bulls. After treatment, Berman claimed an improvement in activity, size, learning, and resistance against infectious diseases. Throughout the following three decades several studies showed a positive correlation between the pineal gland and the immune system, especially the thymus, although contradictory results were also reported. These studies documented a potential endocrine function of the pineal gland on immune system on the basis of two experimental approaches: (a) surgical or functional pinealectomy and (b) association between melatonin production and circadian and seasonal adjustment in the immune system.

Surgical or Functional Pinealectomy

Both surgical and functional pinealectomy directly correlate to weight loss of the main immune organs. Thus, thymuses and spleens from pinealectomized mice, rats, and hamsters are smaller following pinealectomy *(15–18)*. Specifically, pinealectomized BALB/c mice thymuses showed abnormal involution, almost total absence of lymphocytes, and a depletion of lymphoblasts, whereas the major alterations in the spleen were the lack of evident germinal centers and an apparent inactivity of the red pulp. Moreover, a reduction in the size of lymph nodes associated with follicular loss in the outer cortex, a B-dependent area, was noted together with a reduction in the number of lymphocytes in the paracortex, a T-dependent area *(19)*. Pinealectomy of newborn rats is followed by disorganization of thymic structure *(20)*, whereas, in adults, pinealectomy affects the activities of thymic polyamine biosynthetic enzymes such as L-ornithine decarboxylase (ODC) and *S*-adenosyl-L-methionine decarboxylase (SAMD) *(21–23)*. In birds, pinealectomy caused a delay in the development of the chick thymus, spleen, and bursa *(24)*.

Pinealectomy also causes several changes in the immune response. Thus, although the first studies performed in 1970s showed that the pinealectomy caused partial and transient impairment of immune potential in adults rats, whereas no effect were observed after neonatal pinealectomy *(25)*, in recent years, Beskonakli et al. *(26,27)* have found that neonatal pinealectomy significantly influences immune functions, i.e., impairing hematological parameters, including lymphocytes, erythrocytes, and leukocytes, and promoting a deficiency of the brain response to the *Staphylococcus aureus* infection. Continuous light exposure functional pinealectomy in rats causes a reduction in the production of the thymic peptides thymosin α1 and thymulin *(28)* and an enhances α2-adrenergic catecholamine-induced immunosuppression in peripheral blood lymphocytes (PBL) *(29)*. In BALB/c mice injected with sheep red blood cells (SRBC), primary and secondary antibody production was markedly decreased in mice treated with the α-adrenergic receptor antagonist propanolol in the evening *(19)*, while neonatal pinealectomy impaired antibody-dependent cellular cytotoxicity (ADCC) *(30)*. Moreover, an altered zinc turnover and impaired immune functions, such as interleukin-2 (IL-2) plasma levels, were also evident in these pinealectomized animals *(31)*. In C57BL/6 mice the absence of the pineal gland significantly reduces IL-2 production and NK activity *(32,33)*. In other rodents including the Siberian hamster and *Funambulus pennanti*, an Indian tropical rodent, the abolishment of the pineal function reduces the cellular and humoral immune response *(34,35)*. Pinealectomy also affects several immune parameters in birds such as the humoral immune response in chicks *(36)*; non-specific immunity in chickens *(37)*, ring doves *(38)*, and juvenile female fowl *(39)*; and both the humoral and cellular immune response in Japanese quail *(40)*. Immature sheep are the only species in which pinealectomy does not produce noticeable effects in the immune system, at least on IL-2 production, because the reduction of melatonin production fails to lower its production *(41)*. When melatonin was administered to pinealectomized animals, the effects on immune system are typically reversed.

Association Between Circadian Melatonin Production and Adjustments in the Immune System

Because of the rhythmicity observed in some neuroendocrine responses, it is not surprising that some immune parameters also exhibit similar fluctuations. In this context, diurnal and seasonal rhythms of cell proliferation in the mammalian bone marrow and lymphoid system *(42)*, lymphocyte subsets *(43)*, NK activity *(44)*, and cytokine production *(45)* have been described. However, the first indications of a direct relationship between photoperiod and immune system were described in 1973 when two independent studies demonstrated that short days increased both thymus weight in young voles *(46)* and spleen mass in deer mice *(47)*. Several years later, similar results were obtained in Syrian hamsters *(48)*. Since then, numerous studies describing photoperiod effects on the immune system have been published, reaching the general conclusion that short day lengths are usually associated with enhanced immune function *(49)*.

The involvement of melatonin in the photoperiodic control of several immune parameters was revealed for the first time in 1988 when Kuci et al. *(50)* observed that the nocturnal peak of melatonin was closely associated with the proliferation peak of progenitor cells for granulocytes and macrophages (CFU-GM) in C57BL/6JHAN mice *(50)*. Several years later, circadian rhythmicity loss on CFU-GM proliferation was observed in pinealectomized rats *(51)*. Subsequently, a number of reports have confirmed the correlation between nightime melatonin levels with the number and response of immune cells in humans and several rodents *(52–55)*. Furthermore, melatonin seems to play a relevant role in the recovery of immune circadian organization in arthritic rats *(56)*. A close relationship between melatonin and non-specific immunity has also been observed in birds, in which the nocturnal increase in serum melatonin correlates with a rise in phagocytic activity of heterophils, the primary phagocyte present in birds *(57,58)*. Nelson et al. *(59)* have proposed that a long-night pattern of melatonin synthesis enhances immune function to protect against difficult winter conditions, when the low temperature and limited food availability could compromise immune function.

Immunomodulatory Properties of Exogenous Melatonin

In Vivo Melatonin Administration on Immune Response

Since Vaughan et al. *(60,61)* showed that daily afternoon injections of melatonin induced an increase in thymus weight in the gerbil and spleen hypertrophy in the Syrian hamster, most studies published on this subject have confirmed that melatonin administration promotes a clear immunoenhancement in terms of immune tissue morphology. Thus, melatonin causes an increase in the weight of thymus and spleen of several rodents, both under basal conditions *(62)* and in aged-related *(63)* or dexamethasone-induced immunosuppression *(64)*. Administration of melatonin to growing rats provides significant protection against the injurious effects of dexamethasone including a decrease of body weight and atrophy of thymus and adrenals *(65,66)*.

Melatonin administration also increases the proliferative capacity of mouse splenocytes *(67,68)* and rat lymphocytes *(29,69)*. Furthermore, melatonin affects non-specific response in mammals. In mice, melatonin administration promotes an increase in the number of NK cells and monocytes in the bone marrow *(70)* as well as in ADCC, a lytic mechanism in which a specific antibody acts cooperatively with leukocytic effector cells to induce cell lysis *(54,71)*, whereas in humans, Lissoni et al. *(72)* have described a melatonin-induced enhancement in NK activity.

The melatonin effects on the humoral response in non-immunodepressed animals are less clear. Although pineal extracts increase both the number of antibody-forming cells generated and the response against SRBC immunization in mice spleen *(73,74)*, other authors have reported no melatonin effects in mice *(68)* or Syrian hamsters *(75)*. Regarding birds, transient and continuous drinking water administration of melatonin to Japanese quail induces significant increases in the humoral immune responses without prior immunosuppression *(76)*.

An additional function of melatonin in the immune system is the modulation of several immune mediators through regulating their gene expression and production. Thus, melatonin enhances antigen presentation by mouse splenic macrophages to T cells as well as increases both expression of MHC class II molecules and IL-1 and tumor necrosis factor-alpha (TNF-α) production *(77)*. Several pineal indoles upregulate the gene expression of monocyte colony-stimulating factor (M-CSF), TNF-α, transforming growth factor-beta (TGF-β), and stem cell factor (SCF) in peritoneal macrophages as well as the level of IL-1β, interferon-gamma (IFN-γ), M-CSF, TNF-α, and SCF in splenocytes *(78)*. Chronic administration of melatonin for 5 d to antigen-primed mice seems to induce a Th2 cell response through an increase in the IL-10 production and the reduction in TNF-α production *(79)*. In rats, melatonin increases the generation of the thymic peptides thymosin α1 through an increase in the expression of the prothymosin α gene *(28)*, whereas, in the Syrian hamster, melatonin enhances IFN-γ only when is injected in the afternoon *(75)*.

Melatonin also participates in the apoptosis regulation of T *(80)* and B *(81)* cells. Whereas orally administered melatonin inhibits B-cell apoptosis in pre-B-cell stage in mouse bone marrow *(81)*, melatonin exerts anti-apoptotic activity on T cells throughout all stages of their development in thymus of Wistar rats *(80)*.

In general, these publications show that situations in which the immunostimulatory effects of melatonin are best demonstrated are those in which the immune system is depressed. Thus, Maestroni and co-workers *(82,83)* have described the melatonin effects on immunosuppressed mice by propanolol or corticoids, in which melatonin counteracted the decrease on primary antibody response to SRBC and the reduced reactivity against antigens in spleen. These results agree with those obtained by Caroleo et al. *(84–86)* in old or cyclophosphamide-treated mice, where melatonin increased T helper cell activity and IL-2 production. In this context, Maestroni and colleagues *(87)* have postulated that all these melatonin effects may be mediated by an opiatergic mechanism, because the use of naltrexone, a specific opioid antagonist, prevented the melatonin immunoenhancing properties. Moreover, beta-endorphin and dynorphin, which belong to the melatonin-induced opioid system (MIOS), mimic the effects of melatonin *(88–90)*. Wajs et al. *(91)* have also described that late-afternoon injections of melatonin stimulates the expression of the third exon of proopiomelanocortin (POMC), the endorphins α, β, and γ precursor, in the lymph nodes and bone marrow of rats. Recently, an enhancement in cellular and humoral immune responses in the Japanese quail *(92)* as well as an anti-inflammatory effect on experi-

mental peritonitis in chickens *(93)* have also been described via an opiatergic mechanism.

Therefore, these works show an in vivo immunoenhancing action of melatonin that seems most pronounced in those situations in which the immune system is depressed and/or when melatonin is administered in the late-afternoon or evening. These facts would be the reason why other authors have reported no effect of melatonin in mice *(94)*, rats *(95)*, and sheep *(96)*.

In Vitro Effects of Melatonin on the Immune System

Although in vivo models have shown that melatonin may be considered as a positive regulator of immune responses, when melatonin is used in vitro, the results are less clear. In isolated human phytohemoagglutinin (PHA)–stimulated lymphocytes, melatonin in the 1 n*M* range activates T cells through an increase in both the proportion of cells bearing IL-2 receptors (IL-2R) and those carrying T cytotoxic receptors *(97)*. Drazen and co-workers *(98,99)* have also shown that 1 n*M* melatonin markedly increases the proliferation of prairie vole splenocytes in response to the T-cell mitogen, concanavalin A (ConA). The same melatonin concentration also restored a reduction of B lymphocyte proliferation from tonsils of children *(100)*. Doses of melatonin from 10^{-12} to 10^{-6} *M* have also been described as significantly increasing human PBMC proliferation *(101)*.

On the contrary, other in vitro studies found no effect of melatonin on resting or activated lymphocytes with PHA, ConA, or PMA. Thus, melatonin both at low and high concentrations failed to activate the proliferation of human *(102)*, rat *(103)*, or mouse *(104)*. In some cases, an inhibitory effect of melatonin on lymphocyte proliferation has been described as being coupled to inhibition of NK activity *(105)*, IFN-γ and TNF-α production *(106)*, or expression of genes correlated to T lymphocyte activation *(107)*.

The main in vitro melatonin effect studied is the modulation of the immune mediators such as the cytokines. Colombo et al. *(108)* mentioned a direct effect of melatonin on cytokine production. They showed that melatonin increased IFN-γ production by mouse splenocytes, this stimulation being higher in cells isolated at night than in those in the morning. Later, García-Mauriño et al. *(109)* noted that 0.1 n*M* melatonin activated human T cells (Th1 type) by increasing the production of IL-2 and IFN-γ. These results were observed only when cells were isolated in the early afternoon and just slightly activated with PHA. Th2 cells appeared not to be affected by melatonin since IL-4 production, primarily produced by Th2 cells, was not modified. Melatonin (1 n*M*) also increases IL-2 production by Jurkat cells, a human lymphocytic cell line, activated by either suboptimal concentrations of PHA or PMA *(110)*. Finally, melatonin appears to repress 5-lipoxygenase gene expression in human B cells via a nuclear interaction *(111)*. Moreover, melatonin modulates the response of human and mouse monocytes. Melatonin (0.1 n*M*) in combination with lipopolysaccharide (LPS) induces cytotoxicity of human monocytes by increasing IL-1 and reactive oxygen intermediate (ROI) production *(112)*. At same concentration, melatonin activates IL-6 and IL-12 *(109,113)* and decreases IL-10 *(101)* production by PBMC. Both the increase in IL-12 and the reduction in IL-10 production by melatonin can, in turn, act on human T cells causing a Th1-type response. Melatonin also increases TNF production and decreases the release of tissue factor (TF) *(114)*, the most potent trigger of blood coagulation activation *(115)*. Finally, melatonin treatment inhibits the LPS-induced generation of nitric oxide (NO) *(116)* through an inhibition on inducible nitric oxide synthase (iNOS) expression *(117)*.

Melatonin also acts on hematopoietic system where it seems to influence the blood-forming system in mice via the induction of T helper cell–derived opioid cytokines (MIOS system), which exert significant colony-stimulating activity *(118,119)*. These effects of melatonin appear to be mediated by the type 1 κ-opioid receptor on bone marrow macrophages and by IL-1 *(120)*.

The in vitro effects of melatonin on the nonspecific immune response have also been studied. In human neutrophils, low doses of melatonin (10 n*M* range) resulted in an increase of the respiratory burst in response to PMA *(121)*. However, 2 m*M* melatonin inhibited the respiratory burst. Apparently, melatonin modulates this function in a dose-dependent biphasic manner. Recently, a paper has noted that melatonin inhibits LPS-mediated production of TNF-α and IL-8 in neutrophils and surprisingly N^1-acetyl-N^2-formyl-5-metoxykynuramine (AFMK), a melatonin oxidation product recently described in several immune cells *(122)*, was more potent than the melatonin itself *(123)*. In birds, physiological concentrations of melatonin are also able to increase phagocytic activity and reduce superoxide anion levels in heterophils *(124–126)*.

Intra-, Auto-, and/or Paracrine Actions of Immune System Synthesized Melatonin: Nonendocrine Actions

Data collected during the last decade have uncovered numerous functions of melatonin that have caused a change in the classification of melatonin as being exclusively a hormone *(127)*. One of the most evident features that distinguishes melatonin from classical hormones is its synthesis by a number of extrapineal organs, which are regarded conventionally as nonendocrine organs. These include retina *(128)*, Harderian gland *(129)*, gut *(130,131)*, skin *(132)*, and many others where the presence of melatonin or the key enzymes involved in its synthesis have been identified *(10,11,133)*. One of the most obvious sources of extrapineal melatonin is the immune system, where melatonin has been located in thymus and mast cells, NK cells, eosinophilic leukocytes, platelets, and endothelial cells *(10,11)*, as well as several immune cell lines *(134,135)*. In addition, it has

been shown that human PBMCs cultured in the presence of serotonin or IFN-γ can synthesize melatonin *(136,137)*. In recent years, high concentrations of melatonin together with the enzymatic machinery involved in its synthesis have also been described in human, mouse, and rat bone marrow *(138, 139)*. Recently, our group has found that cultured human lymphocytes synthesize and release large amounts of melatonin *(12)*. Furthermore, we have shown for the first time the potential physiological action of this human lymphocyte–synthesized melatonin, which acts as an intra-, auto-, and/or paracrine substance, via its membrane and/or nuclear receptors. These actions are an essential part for an accurate response of human lymphocytes through the modulation of the IL-2/IL-2R system *(140)*. Additionally, Martins et al. *(141)* have shown that macrophages obtained from the peritoneal cavity of normal rats when incubated with tryptophan show an increase in AA-NAT activity that corresponds to an increased melatonin production.

In addition to the direct effect on the regulation of immune system described above, the presence of melatonin in the culture medium released by immune cells could mask, even transform, the effect of exogenous melatonin. This may be one of the reasons that in vitro melatonin effects on the immune system are sometimes contradictory or more contradictory than those in vivo. This may explain why Colombo et al. *(108)* only found a melatonin effect on IFN-γ in splenocytes isolated at night or why García-Mauriño et al. *(109)* only obtained a melatonin effect on the production of several cytokines when cells were isolated in the early afternoon and washed a number of times with saline. The existence of subjects with melatonin-sensitive blood immune cells and the differential response of these cells on the production of LPS-induced TF, depending on season *(114)*, also supports the idea of a masking to endogenous melatonin. Moreover, the presence of endogenous melatonin could also be the reason why many authors are able to find in vitro effects of exogenous melatonin only when cells are not activated or are just slightly activated *(142)*; when cells are fully activated, endogenous melatonin production is either so high or lymphocytes are prepared to respond to this endogenous melatonin, resulting in an inability of exogenous melatonin to have an effect. This fact has been supported by a recent work that used a model in which melatonin synthesis was blocked by para-chlorophenylalanine (PCPA), a reversible tryptophan hydroxylase (TPH) inhibitor; in this case, the in vitro administration of exogenous melatonin significantly increased IL-2 production when human PBMCs were cultured under any condition of PHA stimulation; in the absence of PCPA, exogenous melatonin significantly increased IL-2 production when PBMCs were cultured only in the presence of suboptimal conditions of stimulation *(140)*.

It is also widely known that situations in which stimulatory effects of in vivo melatonin administration on immune system are best demonstrated are those in which the immune system is depressed by aging, physical stress, infectious diseases, treatment with corticoids, or antitumoral or adrenergic drugs *(143)*. Because most of these situations are associated with low levels of endogenous melatonin, this fact once again supports the idea that the effects of exogenous melatonin are influenced by the endogenous levels of indole which would prime the immune system in diverse ways depending on the physiological state of the immune system.

Melatonin Receptors in the Immune System

With recognition that the radioligand 2-[^{125}I]iodomelatonin (^{125}I-Mel) labeled melatonin binding sites, it allowed anatomical localization and pharmacology characterization of melatonin receptors. Initially, ^{125}I-Mel binding sites were classified on the basis of pharmacological and kinetic differences into two subtypes, the ML-1 and the ML-2 melatonin binding sites. The ML-1 binding sites represent high-affinity melatonin receptors with a dissociation constant (K_d) <200 p*M*, and they are coupled to G proteins. A melatonin binding site with low affinity for melatonin (K_d = 0.9–10 n*M*), termed ML-2, was also detected. The ML-2 sites have a distinct pharmacological profile *(144)*.

Regarding membrane melatonin receptors, two mammalian receptor subtypes with high affinity for melatonin have been cloned and characterized, and initially these were termed the Mel1a and Mel1b melatonin receptor *(145,146)*. A third subtype of high-affinity melatonin receptor was cloned from a chicken brain library and termed the Mel1c subtype. However, no mammalian homolog of the Mel1c receptor has been isolated *(147)*. The characteristics of the high affinity "ML-1" receptor were present in each of the three related receptors. Currently, the official nomenclature by the IUPHAR committee dictates that the former Mel1a receptor be designated "mt1" (more recently "upgraded" to "MT_1"), while the Mel1b receptor is designated the MT_2 receptor subtype. The "ML-2" binding site has been re-named the MT_3 melatonin receptor *(148)*. The sequence of the isolated product revealed that the MT_3 site is not a G protein–coupled melatonin receptor, as previously suggested, but instead is due to binding of melatonin to the enzyme quinone reductase (QR2) *(149)*.

An interaction between melatonin and nuclear receptors has also been described. These receptors belong to the RZR/ROR subfamily of nuclear receptors, which includes the products of three genes: splicing variants of RORα (RORα1, RORα2, RORα3, RZRα), which differ in the N-terminal domain, RZRβ, and RORγ *(150)*.

An essential fact that supports the relation between melatonin and the immune system is the presence of melatonin receptors in immune organs and cells. Specific binding sites for melatonin have been described in many immune tissues from a variety species of birds and mammals. Thus, melatonin binding sites have been described in bursa of Fabricius of birds *(151)*; in the spleen of duck *(152)*, chicken *(152,153)*, pigeon *(152,154,155)*, quail *(155)*, guinea pig *(156,157)*,

mouse *(152)*, and rat *(158,159)*; and in the thymus of duck *(160,161)* and rat *(162,163)*. In all these tissues, the K_d values are in the 10–1000 p*M* range.

Specific melatonin binding sites have been also described in humans. Specifically, human PBL binds ^{125}I-Mel with K_d = 1.02 n*M (164–166)*, whereas monocytes have a K_d of 0.27 n*M (167)*. These K_d values suggest that they can recognize both physiological concentrations of melatonin in serum at night and endogenously immune system–synthesized melatonin.

The cellular location of melatonin receptors in the immune system has always been a controversial issue. Although it was historically assumed that melatonin receptors are located exclusively in the plasma membrane of the different immune cells, the presence of nuclear receptors is becoming increasingly evident. In fact, at present, there is sufficient evidence to state that melatonin not only interacts with nuclear receptors but through these sites it exerts several physiological effects on the immune system. This affirmation is based on several main proofs: (a) specific melatonin binding sites have been directly characterized in both the membrane and nucleus. On one hand, membrane melatonin binding sites have been identified in the spleen and thymus of birds *(152–155,161)* and rodents *(156–159, 162,163)*, in mouse peritoneal macrophages *(168)*, and in human lymphocytes *(169)*. Moreover, functional studies have shown that human lymphocyte membrane receptors are coupled to a G protein, and melatonin through these, is able to inhibit forskolin-stimulated cyclic AMP (cAMP) production and cyclic GMP (cGMP) and diacylglycerol (DAG) production *(169)*. Liu and Pang *(151)* found that 50% of the specific binding to bursa of Fabricius homogenate preparations was due to the nuclear fraction, while less than 15% to membranes. One year earlier, Poon and Pang *(156)* had observed binding sites localized in the nuclear fraction (65.5 %) of guinea pig spleens. In the mouse thymuses, Liu et al. *(170)* showed that the subcellular distribution of binding sites was mostly located in the nuclear fraction. When purified cell nuclei of spleen and thymus of rats were studied, it was found that both tissues had melatonin nuclear binding sites, with K_ds of 0.068 and 0.102, respectively *(171)*; (b) in recent years, because of the great advances in molecular biology, both the mRNA and protein of melatonin receptors have been characterized in the immune system. Thus, the expression of MT_1-melatonin receptor mRNA in T and B subsets of lymphocytes from rat thymus and spleen was shown in 1997 *(172)*. Later, García-Mauriño et al. *(110)* reported that Jurkat cells expressed the mRNAs for the nuclear receptors RZRα, RORα1, and RORα2 and for the membrane receptor MT_1, whereas U937 cells, a monocytic cell line, expressed MT_1 or RORα1 and RORα2 mRNAs in the absence or presence of IFN-γ, respectively. The first molecular detection of a human melatonin receptor mRNA was realized in primary cultures of PBMCs, which expressed the MT_1 receptor *(173)*. Subsequent studies confirmed the MT_1 mRNA presence in PBMCs cell populations as well as the presence of RZRα, RORα1, and RORα2 mRNAs *(174)*. Recently, the presence of MT_1 and RORα mRNA and protein has also been detected in both the thymus and spleen of mice, while MT_2 receptor mRNA was also detected, but only in the thymus *(175)*; (c) the development of several specific melatonin membrane and nuclear receptor agonists and antagonists *(176,177)* has allowed the characterization of several physiological roles of both membrane and nuclear receptors in the immune system. Hence, the inhibitory effect of melatonin on forskolin-stimulated cAMP accumulation in mouse peritoneal macrophages is blocked by luzindole, a membrane melatonin receptor antagonist *(168)*. The administration of luzindole either in vitro or in vivo significantly attenuated the ability of in vitro melatonin to enhance splenic lymphocyte proliferation of both wild-type mice *(178)* and MT_1 –/– mice *(179)*, suggesting a direct interaction of MT_2 receptors in the process. Recently, using luzindole and the selective MT_2 blocker, 4P-PDOT, Markowska et al. *(180)* have found that the stimulation of proliferation in chicken splenocyte cultures occurs via Mel1c receptors and is associated with a reduction in cAMP and an increase in IP3 levels, whereas in mitogen-activated splenocytes, melatonin-induced inhibition of proliferation is mediated by MT_2 receptors through an increase of cAMP and a decrease of IP3.

Compounds belonging to thiazolidine diones such as CGP 52608 and CGP 55644 have been used as specific analogs of melatonin nuclear receptors. Thus, CGP 52608, a specific nuclear melatonin receptor agonist, mimics the stimulatory effect of melatonin on IL-2 and IL-6 production by human PBMCs, while the membrane MT_1 receptor agonist S 20098 failed to activate the production of either cytokine *(109,181)*. Similar results were obtained by the same authors when Jurkat cells were used *(110)*. A possible link between nuclear and membrane melatonin receptor on the regulation of the production of cytokines has also been suggested. Thus, García-Mauriño et al. *(181)* observed a synergistic effect of S 20098 and CGP 52608 on IL-6 production by human PBMCs. Later, Carrillo-Vico et al. *(140,173)* showed that both exogenous and endogenous melatonin act on IL-2 production by human PBMCs through either membrane and/or nuclear receptor pools depending on the physiological state of the cell.

Melatonin in Immune System Pathologies

Melatonin and Infection

Evidence of melatonin's ability to protect against viral encephalitis was provided by Maestroni et al. *(88)*, who showed that it prevented paralysis and death in mice infected with encephalomyocarditis virus (EMCV) after acute stress. Ben-Nathan et al. *(182)* also reported that the administration of melatonin reduced viremia and significantly postponed the onset of the disease and death in mice infected with the lethal

Semliki Forest virus (SFV) and attenuated non-invasive West Nile virus (WNV). Similar changes occur in mice infected with Venezuelan equine encephalomyelitis virus (VEEV), where melatonin delays the onset of the disease and reduce mortality *(183–185)*. Zhang et al. *(186)* also 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 in mice with acquired immune-deficiency syndrome (AIDS) caused by LP-BM5 leukemia retrovirus. Melatonin also suppressed the elevated production of Th2 cytokines, reduced hepatic lipid peroxidation, and prevented the loss of vitamin E.

Melatonin and Inflammation

In the last decade, melatonin has also been shown to play an important role in immunopathological conditions such as acute and chronic inflammation *(187–189)*. Septic shock is a systemic response which can be caused by bacterial endotoxins such as LPS which through an interaction with receptors on the surface of a variety of host cells induce the generation of numerous pro-inflammatory factors such as TNF-α, IL-1β, IL-6, IL-12, IFN-γ, and NO *(190)*. Most studies relating to melatonin and endotoxin-induced processes observed that administration of melatonin improves the survival of mice and rats from a lethal dose of LPS with survival rate higher than 80% *(191–193)*. In this context, melatonin has been shown to prevent endotoxic-induced circulatory failure in rats through an inhibition of TNF-α levels *(193)* and to reduce postshock levels of IL-6 in male C3H/HeN mice *(194)*. Moreover, a vast amount of evidence has shown that melatonin counteracts LPS-induced expression of inducible and mitochondrial nitric oxide synthase (iNOS and mtNOS) as well as decrease NO levels in mice and rats *(195,196)*. Furthermore, the melatonin abolishes the LPS-induced rise in lipid peroxidation in both in vivo and in vitro inflammation models *(197,198)*. Furthermore, a clinical study has shown that abnormalities in the circadian melatonin secretion in septic patients are mainly related to the presence of severe sepsis *(199)*. Melatonin also modulates allergic lung inflammation by improving the ability of cells to migrate from the bone marrow to the bronchoalveolar fluid *(200)*. In newborn infants as well, melatonin has been shown to improve clinical outcome and prevent death due to septic shock *(201)*.

Melatonin and Autoimmunity

It must be noted that the pharmacological effect of melatonin on the immune response may not always be beneficial. Thus, the role of melatonin on the autoimmune disease rheumatoid arthritis (RA) seems to be negative, although in others such as multiple sclerosis and lupus its effects are still controversial.

In an autoimmune arthritis model developed in mice and rats, melatonin administration induces a more severe arthritis. Accordingly, pinealectomy reduced the severity of the arthritis by a slower onset of the disease, a less severe course of the disease, and reduced serum levels of anticollagen II antibodies; conversely, melatonin administration exacerbated inflammation in young rats injected with FCA *(202)*. Thus, factors that enhance endogenous melatonin production might play a role in the etiology of RA.

It is interesting to note that the geographical distribution of RA shows a north–south gradient, with higher latitudes being associated with an increased incidence and severity of RA *(203)*. The increased season-associated variability in the photoperiod might mean enhanced melatonin production especially during the long winter nights. In addition, the clinical symptoms of RA show a circadian variation with joint stiffness and pain being more prominent in the early morning. Consistently, human proinflammatory cytokine production exhibits a diurnal rhythmicity with peak levels during the night and early morning at a time when plasma cortisol is lowest *(204)*. Consequently, the clinical symptoms of RA might be related to the circadian rhythm of melatonin synthesis and release.

One clinical study strongly supports a close relationship between melatonin production and IL-12 and NO production by macrophages from RA patients *(205)*. Other authors also found that RA patients have higher nocturnal serum concentration of melatonin than healthy controls. Another interesting observation was that macrophages infiltrating the synovial fluid of RA patients showed specific melatonin binding sites and melatonin was also present at high concentrations in the synovial fluid *(206)*. Although most studies suggest that melatonin might play a promoting role in rheumatoid arthritis, recently it was noted that melatonin administration inhibits the inflammatory response in a model of adjuvant-induced arthritis in rats *(207)*,

Another serious autoimmune disease that might be related to melatonin is multiple sclerosis (MS). As the distribution of people with MS is greater in higher latitudes, shorter winter days could be an environmental factor involved in the etiology of this disease. It has been observed that a melatonin receptor antagonist, luzindole, suppresses experimental allergic encephalomyelitis (EAE), the animal model of MS *(208)*. However, other authors have found that neither winter-type short days nor melatonin supplementation influence the development or severity of the disease *(209)*. Even the beneficial role of melatonin has been described in EAE induced in Lewis rats *(210)*. Moreover, it has been reported that while neonatally pinealectomized Wistar rats develop extensive pathological changes and severe neurologic deficits upon induction of EAE, when rats were pinealectomized at 6 wk of age they appeared resistant to the induction of EAE. This age-related susceptibility suggests that the viral infection that seem associated with the development of MS is most likely acquired in infancy prior to the establishment of the melatonin circadian rhythm (between 3 and 9 mo of age in humans) *(211)*.

The role of melatonin in lupus erythematosus (SLE) is unclear, while the first published work reported a significantly enhancement in the survival of female NZB/W lupus mice when melatonin injections were given in the morning versus afternoon *(212)*, subsequent studies have not observed a clear correlation between disease activity and melatonin levels either in humans and in lupus-prone MRL/MP-fas(Ipr) mice *(213,214)*.

In 2002, Calvo et al. *(215)* published a letter where they hypothesized how melatonin, through the activation of IL-12 by monocytes and the subsequent increase of IFN-γ, exacerbated the symptoms of Crohn's disease in a 35-yr-old woman.

Melatonin, Immune System, and Cancer

Over the last few years, several authors have shown a connection involving neuroimmunomodulatory control loops with the onset and progression of cancer. Cytokines are considered potential immunotherapeutic agents. Currently, some cytokines such as IL-2, IL-4, IL-12, IL-24, IFN-γ, granulocyte-monocyte colony-stimulating factor, and TNF-α are under investigation as cancer therapies *(216)*. Systemic delivery of pharmacological doses of cytokines often results in severe side effects and toxicities because cytokines are relatively unstable in vivo and cancer patients have to receive large amounts of the recombinant protein to maintain the required blood concentration for biological activity. The use of adjuvant immunotherapy has been shown to be an efficient method to diminish these harmful effects, leading to more effective immunotherapy in several kinds of cancer *(217)*. Lissoni's *(218,219)* work over the last 15 yr has shown that the concomitant administration of melatonin with IL-2 amplified the lymphocytosis associated with antitumoral efficiency of IL-2 in several kinds of tumors. Moreover, the simultaneous administration of melatonin enhances the lymphocytosis induced by the IL-2/IL-12 combination and reduces thrompocytopenia (toxicity) levels *(220)*. Some papers have also suggested that melatonin modulates the biological activity and toxicity of TNF-α, another important antitumor cytokine *(221)*.

Melatonin therapy has also been shown to induce decreases in IL-6 levels in patients with advanced solid tumors, which was associated with an improvement in their general well being *(222)*; it also induces a reduction in TNF serum levels *(223)*.

One of the mechanisms that tumors use for evading the immune system is the production of factors which suppress immune Th1 response–mediated cell immunity against tumor cells, promoting a Th2 response *(224)*. Melatonin could counteract this Th2 effect, because it increases IL-12 production by monocytes driving T cell differentiation toward the Th1 phenotype and causing an increase of IFN-γ production *(113)* as well as neutralizing the PGE2 inhibitory actions on IL-2 production *(173)*.

A recent paper has shown a novel therapeutic approach, based on modulation of the neuroendocrine/immune axis through melatonin, as a possible future immunosuppressant in organ transplantation, because melatonin therapy on cardiac graft transplanted rats was able to significantly prolong allograft survival *(225)*.

Concluding Remarks

In this review we have summarized the diverse means that have documented the widespread actions of melatonin in the immune system. Although most of melatonin effects seem to be beneficial, only when we have a complete understanding of the synthesis and actions of melatonin in the immune system will benefits and potential side effects of melatonin become apparent. Melatonin's interactions with the immune system, as currently understood, are summarized in Fig. 1.

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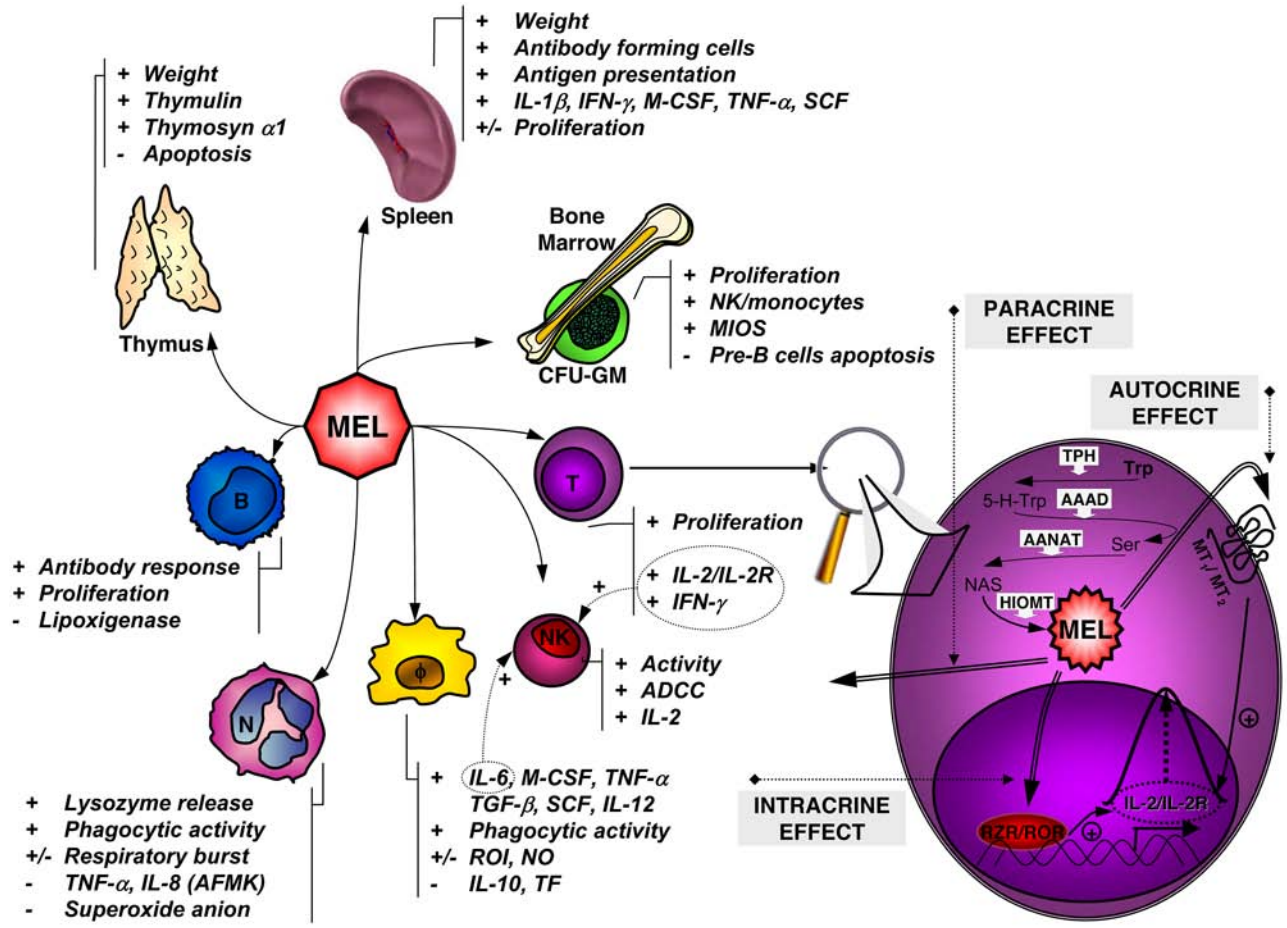

Fig. 1. Hypothetical scheme of melatonin (MEL) effects on the immune system. B cells (B); macrophages (ø); neutrophils (N); T cells (T); 5-hydroxy-triptophan (5-H-Trp); serotonin (Ser); *N*-acetyl-serotonin (NAS).

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