The modulatory role of melatonin on immune responsiveness

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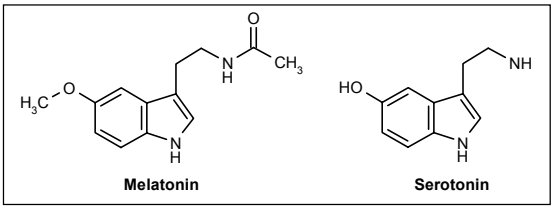
After the successful discovery of the melatonin molecule by Aaron B Lerner et al at Yale University in 1958, melatonin and the pineal gland, a tiny endocrine gland situated at the center of the human brain, have primarily been considered in terms of their effects on the endocrine and reproductive systems. During the last decade, a substantial body of research has defined melatonin as a remarkable molecule with pleiotropic effects on the immune system. Moreover, its synthesis cannot be considered as exclusively endocrine; key immunocompetent cells have the functional enzymatic machinery for melatonin synthesis, paving the way for complex intracrine, autocrine and paracrine regulatory loops. The immunomodulatory role of melatonin, with regard to infection, inflammation and autoimmunity, is outlined here, and the evidence discussed in this review strengthens the notion that the nature of an immune response may be modified, and therefore therapeutically manipulated, by circadian effector signals.

Keywords Extrapineal melatonin, immunopathology, immunotherapy, melatonin, neuroimmunomodulation, vaccines

Introduction

Melatonin (*N*-acetyl-5-methoxy-tryptamine) was the first methoxyindole to be found in mammalian tissue. It was isolated from thousands of bovine pineal glands by Aaron B Lerner [1] in a remarkable attempt to characterize a lightening factor on melanosomes in tadpoles, after initial observations made by McCord and Allen in 1917 [2]. The biosynthesis of melatonin from tryptophan involves four well-defined intracellular steps that are catalyzed by tryptophan hydroxylase, aromatic amino acid decarboxylase, arylalkylamine-*N*-acetyltransferase and hydroxyindole-*O*-methyltransferase [3••]. From a structural point of view, melatonin shares the core structure of serotonin (Figure 1). Melatonin is produced in the pineal gland, under the control of postganglionic sympathetic fibers, and is released during periods of darkness [3••]. This temporal information has consequently been used as a circadian-based photoperiod response and a seasonal timer, and it represents one of the first- and best-characterized physiological roles of melatonin. Furthermore, melatonin demonstrates a remarkable functional versatility by exhibiting antioxidant, oncostatic and anti-aging properties [4••].

Figure 1. The structures of melatonin and serotonin.



The 1990s represented a key period in melatonin research for several reasons. First, the molecular mechanisms of action responsible for its pleiotropic effects were reported, involving several paradigms: high-affinity G-protein-coupled receptors at membrane level, direct interaction with cytosol and nuclear proteins, and both direct radical scavenging of free radical species and redox-modulated processes [5••,6•,7••,8,9,10•]. Second, melatonin was reported in a variety of plant species [11], and as substances normally found in foods do not fall under the jurisdiction of the US Food and Drug Administration melatonin was classed as a dietary supplement with over-the-counter availability, and its use generated great media interest. Third, while the production of melatonin has previously been reported in the retina, Harderian glands and enterochromaffin cells of higher vertebrates, the contribution of extrapineal melatonin remained a matter of debate. The increased number of reports defining the enzymatic machinery of melatonin synthesis and the receptors that can transduce the melatonin message currently highlight the need for considerable research in order to decipher several key issues: (i) which cells endogenously produce melatonin; (ii) under which circumstances this melatonin is released or not; (iii) whether some type of melatonin-concentrating mechanisms exist; and (iv) to what extent extrapineal melatonin is related to the classical circadian-related processes. In this review, we focus on some of the most relevant aspects of melatonin research and its potential impact on the development of new therapeutics, especially those features related to its immunomodulatory actions.

Melatonin and the immune system: Understanding the connection

Pineal-synthesized melatonin is considered to be one of the molecules of the neuro-immune-endocrine axis, a premise that is based on two pioneering experimental approaches: (i) surgical or functional pinealectomy, and (ii) an association

between melatonin production and circadian and seasonal adjustment in the immune system. Surgical and functional pinealectomy are both directly correlated with weight loss and abnormal development in the primary immune organs of mammals and birds [12••,13]. Furthermore, pinealectomy usually leads to a partial or total impairment of immune function, and a number of reports have confirmed the correlation between melatonin levels at night and an enhancement of immune responsiveness (Table 1). When melatonin was administered to pinealectomized animals, the effects on the immune system were typically reverted. Conversely, the association between circadian melatonin production and adjustments in the immune system has been widely observed. The close association between the nocturnal peak of melatonin and proliferation peak of progenitor cells for granulocytes and macrophages has been described in this context [14]. A number of reports have subsequently shown a correlation between melatonin levels at night and the number and response of immune cells in humans, rodents and birds, within the framework of adaptive behavior [15,16•].

Table 1. The effects of pinealectomy on immune response.

Species	Effects	Reference
Rats	Impairment of hematologial parameters and deficiency of the brain response to *Staphylococcus aureus*	[91]
	Decrease in thymosin α1 and thymulin production	[92]
	Enhancing effect on catecholamine-induced immunosuppression in PBL	[93]
Mice	Decrease in primary and secondary antibody production	[94]
	Impairment in ADCC	[95]
	Alteration in zinc turnover	[96]
	Reduction in IL-2 production and NK cell activity	[97]
Other rodents	Reduction on cellular and humoral response	[98]
Birds	Disorders in non-specific, humoral and cellular response	[99,100]
Lambs	No effects on IL-2 production	[101]

ADCC antibody-dependent cellular cytotoxicity, **IL** interleukin, **NK** natural killer, **PBL** peripheral blood lymphocytes.

The immunomodulatory role of exogenous melatonin

The majority of studies published on this subject have confirmed that melatonin administration promotes a clear immuno-enhancement in terms of immune tissue morphology. Thus, melatonin causes an increase in weight of thymus and spleen [17] of rodents, both under basal [18] and immunosuppressive conditions [19].

Melatonin administration also increases the proliferative capacity of mouse splenocytes [20] and rat lymphocytes [21]. Moreover, melatonin affects the non-specific response, promoting an increase in the number of natural killer (NK) cells and monocytes in the bone marrow [22], as well as in antibody-dependent cellular cytotoxicity [23], whereas in humans, a melatonin-induced enhancement of NK activity has been described [24]. Pineal extracts increase both the number of antibody-forming cells generated and the response against sheep red blood cell immunization, with regard to the humoral response, in mouse spleen [25,26]. Administration of melatonin to birds also induces a significant increase in the humoral immune response without prior immunosuppression [27]. An additional function of melatonin in the immune system is the modulation of several immune mediators, via the regulation of their gene expression and production when administered *in vivo* (Table 2). Melatonin also participates in the regulation of apoptosis of T- [28] and B-cells [29]. Overall, these data show that the immunostimulatory effects of melatonin are optimally demonstrated in situations in which the immune system is depressed [15]. Maestroni *et al* have postulated that all of these effects of melatonin may be mediated by an opiatergic mechanism, since the use of naltrexone, a specific opioid antagonist, prevents melatonin from exerting immuno-enhancing properties, while β-endorphin and dynorphin mimic the effects of melatonin [30]. This research demonstrates the immuno-enhancing action of melatonin *in vivo*, which appears to be most pronounced in situations in which the immune system is depressed and/or when melatonin is administered in the late afternoon or evening. This may be the reason why other researchers have reported no effect of melatonin in mice [31], rats [32] and sheep [33].

Table 2. Modulation of immune mediators *in vivo* by administration of melatonin.

Species	Effects	Reference
Mice	Enhancement of antigen presentation by splenic macrophages and increase in MHC-II, IL-1 and TNFα production	[84]
	Upregulation of M-CSF, TNFα, TGFβ and SCF gene expression in peritoneal macrophages as well as of the levels of IL-1β, IFNγ, M-CSF, TNFα and SCF in splenocytes	[102]
	Increase of IL-10 production and reduction in TNFα production	[103]
Rats	Increase in the generation of thymosin α1 through an increase in prothymosin α gene expression	[92]
Hamster	Enhancement of IFNγ production	[104]
Sheep	Adjuvant-like properties and enhancement of antibody production	[83]

IFN interferon, **IL** interleukin, **M-CSF** macrophage-colony stimulating factor, **MHC** major histocompatibility complex, **SCF** stem cell factor, **TGF** transforming growth factor, **TNF** tumor necrosis factor.

The findings are more controversial when melatonin is investigated *in vitro*. In the 1-nM range, melatonin activates phytohemoagglutinin (PHA)-stimulated human T-cells through an increase in both the proportion of cells bearing interleukin (IL)-2 receptors (IL-2Rs) and those carrying T-cytotoxic receptors [34]. Furthermore, melatonin increases the proliferation of prairie vole splenocytes in response to the T-cell mitogen concanavalin A (ConA) [35], and counteracts the reduction of B-lymphocyte proliferation in

tonsils from children [36]. Conversely, other studies found that melatonin had no effect on resting or activated lymphocytes using PHA, ConA or phorbol 12-myristate 13-acetate. In some cases, an inhibitory effect of melatonin has been observed [15].

The most studied *in vitro* effect of melatonin is the modulation of immune mediators such as cytokines (Table 3). Melatonin also acts on the hematopoietic system, where it appears to influence the blood-forming system in mice via the induction of T-helper cell (Th)-derived opioid cytokines (the MIOS system), which exert significant colony-stimulating activity [37]. The *in vitro* effects of melatonin on the innate immune response have also been studied. In human neutrophils, melatonin modulates the respiratory burst [38], while melatonin and its oxidation product N^1-acetyl-N^2-formyl-5-methoxykynuramine, which has been described in several immune cells [39], inhibit lipopolysaccharide (LPS)-mediated production of tumor necrosis factor (TNF)α and IL-8 in neutrophils [40]. Physiological concentrations of melatonin can also increase phagocytic activity and reduce superoxide anion levels in heterophils in birds [41].

Local actions of immune-system-synthesized melatonin

Pioneering research has demonstrated direct evidence of melatonin biosynthesis from tryptophan on immunocompetent cells within the context of an immunodulatory circuit [42••,43•]. The cells and organs of the immune system are remarkable sources of melatonin, and concentrations of melatonin and/or the enzymatic machinery involved in its synthesis have been determined in human, mouse and rat bone marrow [44,45•], as well as in human immunocompetent cells [46••,47•]. It has been found that cultured human lymphocytes synthesize and release large amounts of melatonin [46••], which acts as an intra-, auto- and/or paracrine substance, via the modulation of the IL-2/IL-2R system by its membrane and/or nuclear receptors [48••]. In addition, macrophages obtained from the peritoneal cavity of normal rats can produce melatonin after incubation with tryptophan [49].

When cells are fully activated, the endogenous melatonin overproduction results in an viability to sense exogenous melatonin [48••]. Alternatively, activated cells may not respond to exogenous melatonin because of specific downregulation mechanisms of the melatonin of receptor-effector system, as has been suggested [51•]. Thus, the fact that endogenous melatonin synthesis by human peripheral blood mononuclear cells is involved in the IL-2/IL-2R system [48••,51] places melatonin and its metabolites as putative key elements that fine-tune the immune system accordingly.

Melatonin receptors in the immune system

The mechanistic link that supports melatonin as a direct biological response modifier of the immune system is based on the presence of melatonin receptors in the immune organs and cells (Table 4). Early reports revealed the presence of melatonin binding sites in the immune system, both in membrane and nuclear fractions (for an extensive review see references [15,52]). Functional studies proved that human lymphocyte membrane receptors are coupled to a G-protein-signaling system [53], while the cloning of melatonin receptors [54••] led to the first disclosure of mRNA gene expression in the immune system [55•]. Melatonin has also been reported to interact with the RZR/ROR subfamily of nuclear receptors (extensively reviewed in reference [9]), which are present in different rodent and human immunocompetent cells [47•,56•]. The development of several specific melatonin membrane and nuclear receptor agonists and antagonists [57••,58] has allowed the characterization of several physiological roles for both membrane and nuclear receptors in the immune system [48••,59].

Melatonin in immune intervention

Melatonin and infection

Melatonin reduces mortality and delays the onset of disease in several viral infections induced by encephalomyocarditis virus, lethal Semliki Forest virus, non-invasive West Nile virus and Venezuelan equine encephalomyelitis virus [60]. Treatment with melatonin alone, or in combination with dehydroepiandrosterone, also prevents the reduction of B- and T-cell proliferation as well as Th1 cytokine secretion in mice with AIDS [61]. Melatonin also suppressed the elevated production of Th2 cytokines, reduced hepatic lipid peroxidation and prevented the loss of vitamin E in this model.

Melatonin and inflammation

In the last decade, melatonin has been shown to improve the survival rate of mice and rats after administration of a lethal dose of LPS [62], through the inhibition of pro-inflammatory factors, such as cytokines and nitric oxide, as well as to decrease lipid peroxidation levels and apoptosis [63]. One clinical study has revealed a relationship between abnormalities in circadian melatonin secretion in septic patients and the presence of severe sepsis [64]. Furthermore, in a study conducted in newborn infants, melatonin improved clinical outcome and prevented death due to septic shock [65].

Table 3. The primary effects of melatonin on immune mediators *in vitro*.

Species	Cell	Effects	Reference
Human	PBMCs/T-cells	Increase in IL-2, IFNγ and IL-12 and reduction in IL-10 production	[81]
	Monocytes/macrophages	Increase in IL-1 and ROI production	[105]
		Activation of IL-6, IL-12 and TNFα and decrease in IL-10 production	[50,81,106]
	B-cells	Repression of 5-lipoxygenase gene	[107]
	Jurkat	Enhanced IL-2 production	[108]
Mice	Monocytes/macrophages	Inhibition of LPS-induced NO production	[109]
	Splenocytes	Increase in IFNγ production	[110]

IFN interferon, **IL** interleukin, **LPS** lipopolysaccharide, **NO** nitric oxide, **ROI** reactive oxygen intermediate, **TNF** tumor necrosis factor.

Table 4. Melatonin receptor presence in the immune system.

Location	Species	Organ/cell	Receptor	Putative mechanism of action	Approach	Reference
Membrane	Human	PBMCs	MT_1	Modulation of IL-2 production through decreasing cAMP levels	Agomelatine, membrane agonist	[111•]
		Jurkat cells	MT_1 MT_2	IL-2 regulation	Luzindole, membrane antagonist	[51]
		U937 cells	MT_1			[108]
	Mouse	Thymus	MT_1 MT_2		Agomelatine	[56•]
		Spleen	MT_1			
		Peritoneal macrophages		Inhibition of forskolin-stimulated cAMP accumulation	Luzindole	[48••]
		Splenic lymphocytes	MT_1 MT_2	Enhancement of proliferation through MT_2	Luzindole	[112]
	Rat	Thymus	MT_1			[55•]
		Spleen	MT_1			
	Chicken	Splenocytes	Mel_{1c}	Stimulation of proliferation via reduction in cAMP and increase in IP_3 levels	Luzindole, luzindole and 4P-PDOT, MT_2-specific antagonist	[113]
			MT_2	Inhibition of proliferation in mitogen-activated cells through increase of cAMP and decrease of IP_3	Luzindole, luzindole and 4P-PDOT, MT_2-specific antagonist	[113]
Nuclei	Human	PBMCs	RZRα RORα1 RORα2	Cytokine production and regulation		[47•,48••,81]
		Jurkat cells	RZRα RORα1 RORα2	Regulation of IL-2 levels	CGP-52608 CGP-55644	[51,108]
		U937 cells	RORα1 RORα2	IL-6 production	CGP-52608	[108]
	Mouse	Thymus	RORα			[56]
		Spleen	RORα			

IL interleukin, **IP3** inositol triphosphate. Empty boxes means function/mechanism of action is not known.

Melatonin and autoimmunity

It should be noted that the pharmacological effect of melatonin on the immune response may not always be beneficial. Thus, the role of melatonin with regard to the autoimmune disease rheumatoid arthritis (RA) appears to be negative, although in other such disorders, for example, multiple sclerosis (MS) and lupus, its effects are controversial. In an autoimmune arthritis model that has been developed in mice and rats, both melatonin administration and pinealectomy induced a more severe arthritis [66,67]. Geographical distribution of RA shows that higher latitudes are associated with an increased incidence and severity of RA [68]. 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. Human pro-inflammatory cytokine production consistently exhibits a diurnal rhythm, with peak levels during the night and early morning at a time when plasma cortisol is low [69••]. Thus, the clinical symptoms of RA might be related to the circadian rhythm of melatonin synthesis and release. An interesting observation is that macrophages infiltrating the synovial fluid of RA patients showed specific melatonin binding sites, and melatonin is also present at high concentrations in synovial fluid [70].

MS might also be related to melatonin. The distribution of people with MS is greater in higher latitudes, so shorter winter days could be an environmental factor that is involved in the etiology of this disease. It was observed that a melatonin receptor antagonist, luzindole, suppressed experimental allergic encephalomyelitis (EAE), the animal model of MS [71]. However, other researchers have found that neither winter-type short days nor melatonin supplementations influenced the development or severity of the disease [30]. A beneficial role for melatonin has been described in ameliorating EAE that has been induced in Lewis rats [72].

The role of melatonin in SLE is unclear. Although one study reported a significant enhancement in the survival of female New Zealand Black/White lupus mice when melatonin injections were administered in the morning versus the afternoon [73], subsequent studies have not reported a clear correlation between disease activity and melatonin levels either in humans or in lupus-prone MRL/MP-fas(Ipr) mice [74].

Melatonin, the immune system and cancer

Cytokines are considered to be potential immunotherapeutic agents. Some cytokines, such as IL-2, IL-4, IL-12, IL-24, IFNγ, granulocyte-monocyte colony-stimulating factor and TNFα are currently under investigation as cancer therapies [75]. The use of adjuvant immunotherapy is an efficient method of diminishing the harmful effects associated with the systemic delivery of pharmacological doses of cytokines, leading to more effective immunotherapy in several types of cancer [76]. Over the last 15 years, several studies have shown that the concomitant administration of melatonin with IL-2 amplified the lymphocytosis associated with the antitumoral efficiency of IL-2 in a variety of tumor types. Moreover, the simultaneous administration of melatonin enhanced the lymphocytosis induced by an IL-2/IL-12 combination and reduced thrompocytopenia (toxicity) levels. Some studies have suggested that melatonin modulates the biological activity and toxicity of TNFα, another important antitumor cytokine [77]. Melatonin therapy also induced decreases in IL-6 levels in patients with advanced solid tumors, which was associated with an improvement in their general wellbeing [78]; it also induced a reduction in TNFα serum levels [79]. One of the mechanisms that tumors use for evading the immune system is the production of factors that suppress immune Th1-response-mediated cell immunity against tumor cells, promoting a Th2 response [80]. Melatonin could counteract this Th2 effect, since it increases IL-12 production by monocytes, driving T-cell differentiation toward the Th1 phenotype and causing an increase of IFNγ production [81].

Melatonin as an adjuvant in vaccination

Special attention is being paid to adjuvants capable of efficiently promoting a Th1-type of immune response, which is the best correlate of a protective immune response against infection [82]. A new feature of melatonin as an adjuvant-like system in an open-field vaccination procedure has been reported [83•,201], which is the first report of melatonin-based enhancement of a defined immune response *in vivo*. Two primary mechanisms of action, involving humoral and cellular immune responses, may potentially explain the adjuvant feature of melatonin. First, melatonin could effectively augment the antibody response by enhancing antigen presentation. Previous findings in immunodepressed mice showed that melatonin increased antigen presentation and expression of major histocompatibility complex (MHC) class II molecules by splenic macrophages, leading to enhanced humoral response [84•,85]. Second, melatonin could modulate cytokine production that is critical to the initiation of the immune response and the establishment of cellular regulatory networks. It appears that the adjuvant mechanism of aluminium hydroxide facilitates a Th2 induction, independent of IL-4 and IL-13 Th2 cytokines. This effect constitutes a major limitation in the application of adjuvants derived from aluminum in modern vaccines [86]. Remarkably, it has been reported that co-adsorption of IL-12 to aluminum-based adjuvants elicits a bias in the aluminum-induced response from Th2 to Th1 [87]. Melatonin can promote a Th1 response by increasing the production of IL-12 in human monocytes *in vitro* [88]. The reported correlation between endogenous melatonin and serum IL-12 levels is of physiological relevance [89]. Thus, by inducing IL-12, melatonin enhances the adjuvant properties of aluminum hydroxide and improves its clinical use by selective facilitation of the Th1 response. Moreover, studies aimed to determine the IFNγ to IL-10 ratio as a marker of Th1 and Th2 responses in whole human blood showed a bias toward Th1 responses during the night and early morning when the IFNγ/IL-10 ratio is high [90]. Thus, we raise the possibility that melatonin could be considered in further studies to design novel adjuvant systems.

Conclusion

In evolutionary terms, melatonin is an old modulator, and most probably its antioxidant properties resemble its older evolutionary functions; first, being an intracellular signal and subsequently a hormone with recognized immunomodulatory capabilities. Future identification of a relevant working model of the mechanism of action of melatonin should address the role of endogenous extrapineal melatonin produced by the immune system. Melatonin could be thought of as a safer substance bearing in mind the numerous side effects of many drugs that are already approved in the US and Europe. Two phase I clinical studies are currently underway to examine the effect of melatonin on jet lag symptoms and seasonal affective disorders (NLM identifiers NCT00097474 and NCT00016666: www.clinicaltrials.gov), while a phase II trial has been completed and is in the recruiting stage to determine the effect of melatonin, used as radiosensitization/radioprotection, on overall survival and clinical deterioration in patients with brain metastases (NCT00031967). Nevertheless, it should be stressed that diverse clinical trials are necessary in order to assess the potential of melatonin as an immune modifying agent.

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