Anti-inflammatory effects of melatonin in multiple sclerosis

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Melatonin is a hormone with complex roles in the pathogenesis of autoimmune disorders. Over the years, it has become clear that melatonin may exacerbate some autoimmune conditions, whereas it alleviates others such as multiple sclerosis. Multiple sclerosis is an autoimmune disorder characterized by a dysregulated immune response directed against the central nervous system. Indeed, the balance between pathogenic $CD4^+$ T cells secreting IFN-γ (T_H1) or IL-17 (T_H17); and $FoxP3^+$ regulatory T cells and IL-10^+ type 1 regulatory T cells (Tr1 cells) is thought to play an important role in disease activity. Recent evidence suggests that melatonin ameliorates multiple sclerosis by controlling the balance between effector and regulatory cells, suggesting that melatonin-triggered signaling pathways are potential targets for therapeutic intervention. Here, we review the available data on the effects of melatonin on immune processes relevant for MS and discuss its therapeutic potential.

Keywords:
melatonin; MTNR1A; multiple sclerosis; ROR-α; TH17 cells; Tr1 regulatory cells

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Abbreviations:
CNS, central nervous system; **MS,** multiple sclerosis.

Introduction

Melatonin or N-Acetyl-5-methoxytryptamine is a hormone secreted by the pineal gland during nighttime. The existence of melatonin was first suspected in 1917 in experiments showing that frog melanocytes become lighter when exposed to pineal extracts, and it was formally demonstrated in 1958 by Lerner and colleagues [1, 2]. Melatonin has an important role in the regulation of the circadian rhythm, sleep, and mood, but additional evidence supports its role in immunomodulation, reproduction, tumor growth, and aging [3–6].

The pineal gland is composed by two types of cells: pinealocytes (which produce mostly melatonin) and neuroglial cells [7]. Photic information from the retina is transmitted to the pineal gland through the suprachiasmatic nucleus of the hypothalamus and the sympathetic nervous system. The synthesis and release of melatonin is stimulated by darkness and inhibited by light. The biosynthesis of melatonin begins with the hydroxylation of L-tryptophan into serotonin by tryptophan hydroxylases (TPH) 1 and 2. Serotonin is then converted by the arylalkylamine N-acetylatransferase (AANAT) to N-acetylserotonin, which is further processed to melatonin by the hydroxyindole O-methyltransferase (HIOMT). Melatonin is readily secreted into the bloodstream and cerebrospinal fluid (CSF) as a result of its lipophilic nature [8]. In humans, melatonin secretion increases soon after the onset of darkness and peaks in the middle of the night. In normal adults, the average melatonin levels are 10 pg/mL in the daytime, and 60 pg/mL during the nighttime peak [3–7]. In addition to the circadian oscillation, melatonin also displays seasonal fluctuations that reflect the shortening decrease in daylight during fall and winter [7, 9].

Of note, the pineal gland is not the only site of melatonin synthesis, and may not even be the largest producer: the gastrointestinal tract contains up to 400 times more melatonin than the pineal gland [8, 10]. In addition, bone marrow cells, astrocytes, macrophages, T cells, fibroblasts, and skin cells have also been shown to produce melatonin [11]. However, the physiological relevance of these "peripheral" sources of melatonin is mostly unknown.

Melatonin is remarkably conserved during evolution, even in species that are not considered photoperiodic, such as humans [12]. Based on the high evolutionary conservation of

melatonin production by the pineal gland and its regulation by daylight [12], it is tempting to speculate that the circadian and seasonal effects of melatonin played an important role that drove its positive selection during evolution. Indeed, there is evidence that sunshine time duration placed a selective pressure on melatonin receptor 2, as the rs4753426C allele is inversely correlated with sunshine hours, suggesting that this allele may have indirectly increased survival and reproductive success [13]. For example, increased mortality and incidence of several conditions such as heart, respiratory, and infectious diseases are detected during the winter [14]. Thus, it can be speculated that melatonin contributes to physiological seasonal adjustments that facilitate survival despite changes in ambient temperature, food availability, and other environmental conditions. Consequently, it is possible that melatonin also adjusts the immune response during seasonal fluctuations. Indeed, support for this hypothesis was provided by initial reports which highlighted the "immune boosting" properties of melatonin [15].

Over the years, however, the relationship between melatonin and the immune system was found to be more complex than simply boosting or blocking the immune response. Indeed, melatonin seems to ameliorate some autoimmune diseases, such as multiple sclerosis (MS) and ulcerative colitis (UC) [16, 17], while it may not affect or even be detrimental for others, such as rheumatoid arthritis (RA) [18, 19]. Thus, our understanding of the effects of melatonin on the immune response and autoimmune pathology is still limited. In this review, we discuss recent findings on the complex relationship between melatonin and autoimmune inflammation in MS.

Melatonin signals through several pathways

Most of the biological effects of melatonin have been shown to be mediated by G protein-coupled receptors [3–6]. Melatonin activates two membrane receptors, MT1 (encoded by MTNR1A) and MT2 (encoded by MTNR1B) [6]. MT1 and MT2 differ not only in their structure, but also in their localization: MT1 is highly expressed in the suprachiasmatic nucleus, hippocampus, nucleus accumbens, amydgala, substantia nigra, hypothalamus, and cerebellum; whereas MT2 is expressed preferentially in the retina and to lesser degree in hippocampus and cerebral cortex. In addition to this central expression, MT1 is also expressed at peripheral sites, such as bone marrow, and the immune system [20]. A third membrane receptor was originally described, and named MT3. However, MT3 was later found to be quinone reductase II, an enzyme that participates in the protection of the cell against oxidative stress [21].

Melatonin-triggered MT1 activation modulates a myriad of signaling pathways. Melatonin blocks cAMP synthesis, protein kinase A activity, and the biding of cAMP-responsive elements. On the other hand, MT1 activation leads to the phosphorylation of ERK1/2, MEK1/2, and JNK. MT2 activation is reported to have similar biological effects, such as increased cAMP production and JNK phosphorylation [22].

Intracellular melatonin receptors were also described, but they were later identified as calmodulin, calreticulin, and tubulin, and their contribution to the biological effects of melatonin is largely unknown [20]. Melatonin was also reported as a high-affinity ligand for the retinoid orphan receptor alpha (ROR-α) [23], but this finding remained controversial [24]. Additional evidence accumulated over the years suggest that melatonin is a ROR-α ligand of moderate-affinity as indicated by: (i) molecular modeling studies [25]; (ii) melatonin and ROR-α co-immunoprecipitation [26]; and (iii) melatonin activation of ROR-α responsive reporters in luciferase assays [16, 27]. We believe that two confounding factors may have contributed to the discrepancies reported with respect to the ability of melatonin to activate ROR-α: On the one hand, cholesterol, vitamin D, and other biological molecules usually present in tissue culture media are high affinity ligands for ROR-α [25], thus concentrations of these natural ligands constitute confounding factors in studies aimed at detecting ROR-α activation by melatonin. On the other hand, we [16] and others [28] have shown that signaling by the melatonin membrane receptor affects ROR-α activity.

Besides its interactions with membrane and nuclear receptors, melatonin has non-receptor-mediated effects in the immune system due to its ability to scavenge free radicals [29]. During the metabolism of oxygen (0_2), a small percentage (1–4%) is converted to reactive oxygen intermediaries and free oxygen radicals. The toxic properties of these reactive oxygen species led to their association with tissue damage and aging. Melatonin has been shown to prevent tissue damage by free radicals in several ways. First, melatonin counteracts high toxic hydroxyl radicals (ŸOH) produced during oxygen metabolism [30]. Moreover, melatonin has also been shown to scavenge oxygen byproducts produced by myeloperoxidase (MPO) expressed by macrophages and microglia [31]. Thus, melatonin neutralizes the deleterious effects of reactive oxygen species, and consequently, the resulting activation of inflammatory mediators such as NF-κB at brain lesions in MS [29].

Melatonin modulates the autoimmune response

Autoimmune disorders affect approximately 5–10% of the western human population. This incidence has increased sharply in the past 50 years, a rise that cannot be explained by changes in genetic factors [32]. Because the majority of autoimmune disorders are chronic, they impose a great burden on the individual's quality of life and the health care system.

The strongest evidence supporting a role for melatonin in immune modulation was provided by the observation that pinealectomized mice show reduced thymuses, spleens, and lymph nodes, together with signs of immune deficits [4]. However, the effects of melatonin on the immune response are contradictory, ranging from boosting to suppressing the immune response [33–36] and suggesting that melatonin exerts complex cell- and disease-specific actions. We will

Table 1. Summarized evidence on melatonin and autoimmune diseases

Autoimmune disease	Population/animal model	Intervention	Outcome/results	Reference
Type 1 diabetes	Spontaneous diabetes in NOD mice	Melatonin 4 mg/kg	Decreases disease incidence	[40]
	Spontaneous diabetes in NOD mice	Melatonin 200 mg/kg	Favors islet graft survival	[116]
	Spontaneous diabetes in NOD mice	Pinealectomy	Increases disease incidence	[40]
Rheumatoid arthritis	CIA in DBA/1 mice	Melatonin 1 mg/kg	Worsens arthritis	[51]
	AA in SD rats	Melatonin 1–100 μg/kg	Ameliorates arthritis	[52]
	CIA in rats	Melatonin 30 μg/mouse	No clinical effect, increases IL-1β and IL-6	[117]
	CIA in DBA/1 mice	Melatonin 10 mg/kg	Worsens arthritis	[118]
	CIA in DBA/1 mice	Darkness	Worsens arthritis	[49]
	CIA in DBA/1 mice	Pinealectomy	Ameliorates arthritis	[51]
Inflammatory bowel disease (ulcerous colitis, Crohn's disease)	DSS-induced colitis in mice	Melatonin 150 μg/kg	Ameliorates colitis	[42]
	DSS-induces colitis in mice	Melatonin 2–8 mg/kg bw/day	Improves mucosal healing	[119]
	DSS-induced colitis in sleep-deprived mice	Melatonin 10 mg/kg	Ameliorates colitis	[120]
	DNBS-colitis in rats	Melatonin 15 mg/kg	Ameliorates colitis	[45]
	DNBS-colitis in rats	Melatonin 15 mg/kg	Ameliorates colitis	[121]
	DNBS-colitis in rats	Melatonin 15 mg/kg	Ameliorates colitis	[122]
	TNBS-colitis in rats	Melatonin 5–10 mg/kg	Ameliorates colitis	[44]
	TNBS-colitis in rats	Melatonin 2.5–10 mg/kg	Ameliorates colitis	[123]
	TNBS-colitis in rats	Melatonin 2.5–10 mg/kg	Ameliorates colitis	[124]
	TNBS-colitis in Wistar albino rats	Melatonin 10 mg/kg	Ameliorates colitis	[125]
	TNBS-colitis in rats	Melatonin 2.5–10 mg/kg	Reduces colonic lesions and improves colitis symptoms	[126]
	TNBS-colitis in Wistar rats	Melatonin 0.5–2 mg/kg	Short-term administration ameliorates colitis, chronic administration worsens colitis	[127]
	Acetic acid-induced colitis in rats	Intracolonic gel with melatonin	Improves colonic epithelization	[128]
	Acetic acid-induced colitis in rats	Melatonin 100 mg/kg	Ameliorates colitis	[129]
	Acetic-acid colitis in Wistar rats	Melatonin 10 mg/kg	Ameliorates colitis	[43]
Systemic lupus erythematous	MRL/MpJ-*lpr*^{Fas} mice	Melatonin 30 mg/kg	Ameliorates diseases in females, aggravates disease in males	[54]
	MRL/MpJ-*lpr*^{Fas} mice	Melatonin 30 mg/kg, testosterone, estradiol	Decreases autoantibodies titers	[130]
	Pristane-induced lupus in Balb/c mice	Melatonin 0.01–1 mg/kg	Ameliorates disease	[55]
	Membranous nephritis in Balb/c mice	Melatonin 20 mg/kg	Ameliorates glomeruli inflammation	[131]

focus on the effects of melatonin in MS, referring briefly to other autoimmune disorders. In Table 1, we summarize the available evidence on the effects of melatonin on autoimmune diseases.

Type 1 diabetes (T1D) results from the autoimmune destruction of insulin-secreting β cells in the pancreas. Non-obese diabetic (NOD) mice spontaneously develop autoimmune T1D driven by islet reactive $CD4^+$ T cells [37, 38], with a higher disease incidence in females [39]. Pinealectomy worsens NOD T1D [40]. Melatonin is thought to suppress NOD T1D through both its ability to act as an oxygen radical scavenger, and also its multiple effects on the immune system.

Inflammatory bowel diseases (Crohn's disease and ulcerative colitis) are autoimmune diseases characterized by the chronic inflammation of the digestive tract [41]. Several reports demonstrated a beneficial effect of melatonin in

experimental colitis [42–45], encouraging Chojnacki and colleagues to evaluate the therapeutic effects of melatonin in a human clinical trial on IBD patients [17]. The study included 60 patients, divided in two equal groups of 30 IBD patients each, treated with mesalazine in combination with melatonin or placebo at bedtime for 12 months. The researchers reported that all the patients treated with melatonin remained in remission during the 12 month observation period, whereas the placebo group had significantly higher disease scores after 6, 9, and 12 months. Of note, two independent publications reported that one patient with Crohńs disease and one with ulcerative colitis experienced clinical worsening of IBD upon treatment with melatonin [46, 47]. Overall, these results suggest that melatonin might be helpful as a combination therapy in IBD (Table 1).

Rheumatoid arthritis (RA) is a chronic autoimmune disorder that typically affects small joints and produce significant disability [48]. RA patients suffer from morning stiffness and symptom aggravation, suggesting a possible relationship with circadian factors. The most commonly used animal model of RA is the collagen-induced arthritis (CIA). In this model, constant darkness produces a worsening of the disease, manifested as a higher titer of anti-collagen antibodies and enlarged spleens. In accordance with this finding, melatonin treatment exacerbated, and pinealectomy ameliorated, the development of CIA [49–51]. Of note, one report showed conflicting data in which melatonin improved CIA [52]. Thus, animal model studies suggest that melatonin may have a detrimental effect in RA. Despite these conflicting data, a small clinical trial tested the effects of melatonin in RA patients [18, 19]. The study, which included 75 patients, detected an increase in the Erythrocyte Sedimentation Rate, and concentration of kynurenine and neopterin in treated patients compared with controls. These results suggested a proinflammatory action, but there were no significant effects of melatonin on clinical assessments or the concentration of blood IL-1β, IL-6, and TNF-α.

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune condition characterized by a variety of clinical manifestations that follow a relapsing-remitting course. A hallmark of SLE is multi-systemic inflammation accompanied by autoantibody production [53]. Melatonin has therapeutic effects in SLE animal models. Jimenez-Caliani and colleagues reported that daily melatonin administration reduced autoantibodies levels, decreased inflammatory cytokines, and increased the production of anti-inflammatory IL-10 in MRL-*Fas*^{lpr} females [54]. Melatonin was also therapeutic in the model of pristane-induced lupus: Treatment with various doses of melatonin reduced anti-ssDNA and anti-histone antibody titers and renal injury [55]. Recently, Robeva and colleagues reported that daily melatonin levels are decreased in women with SLE [56]. They also reported an inverse correlation between melatonin and SLE disease activity, suggesting that reduced circulating melatonin levels may contribute to SLE pathogenesis.

All in all, these findings suggest that the modulation of melatonin signaling by the administration of melatonin or specific receptor agonists may provide a therapeutic approach for some immune mediated diseases.

Melatonin in the control of the immune response in MS

MS is a complex immune-mediated disease of the central nervous system (CNS) and is considered one of the leading causes of physical disability in young adults [57]. MS prevalence varies geographically between 0.83 and 40 cases per 100,000 inhabitants in South America and Africa [58, 59] to more than 100 cases per 100,000 inhabitants in most countries of Europe, Canada, and the United States [60]. MS, therefore, affects an estimate of 2.5 million people worldwide representing a major personal and socioeconomic constraint: the typical age of onset is around 30, and up to 50% of such patients will require a wheelchair after 25 years of the disease. The clinical presentation of MS can be variable, including visual and sensory disturbances, motor and coordination impairments, as well as spasticity, pain, fatigue, and cognitive malfunctions [57]. Clinical signs and symptoms are related to the areas of the brain, optic nerve, and spinal cord affected by the infiltration of immune cells that produce demyelination, axonal loss, and disrupt normal signaling between neurons [61]. The majority of patients suffer from relapses or exacerbations followed by a complete or partial recovery, a disease subtype known as relapsing-remitting MS. A minor group of patients experience a steady and continuous deterioration of their symptoms from disease onset (primary progressive) or after several years of relapsing remitting disease (secondary progressive disease).

$CD4^+$ T_H1 cells and T_H17 cells are considered important contributors to MS pathogenesis because they appear early during the formation of CNS lesions [62–64]. Indeed, therapies targeting T_H17 cells are currently being tested in MS with preliminary encouraging results [65]. $CD8^+$ T cells are also present in MS lesions, sometimes at a higher frequency than $CD4^+$ T cells, and their numbers are correlated with the amount of axonal damage [66]. In addition to T cells, clonally expanded B cells can be found in the CSF of MS patients. Indeed, a widely recognized biomarker in MS is the presence of immunoglobulins secreted by clonally expanded B cells, known as oligoclonal bands (OCBs) [67]. $FoxP3^+$ regulatory T cells (Tregs) and IL-10 secreting type 1 regulatory T cells (Tr1 cells) are key regulators of effector T cells: accordingly, deficits in Tregs and Tr1 cells have been described in MS [68–70]. $CD8^+$ T cells and B cells can also display regulatory activity and have been linked to positive responses to immunotherapy in MS [61]. In summary, a fine balance between effector and regulatory T cells controls MS disease activity [62–64].

Genetic polymorphisms have been associated with MS risk and/or pathogenesis [71, 72]. The strongest risk allele for MS is the HLA-DRB1*1501 allele, which is associated with a sixfold higher than mean risk in homozygous carriers. Other HLA alleles, as well as non-HLA loci, were later found associated with a modest risk increase [73]. The fact that genetic factors only explain some of the MS cases, combined with observations on its geographical distribution, led to the search for additional factors linked to disease pathogenesis. As a result, environmental factors such as infections [74–76], sodium intake [77], smoking [78], and vitamin D levels [79] have been proposed to affect MS development and course.

Support for seasonal-occurring disease-modifying factors in MS

The majority of MS patients suffer from relapses in which neurological signs and symptoms occur in relationship with lesions in the brain and spinal cord. In a theoretical scenario where relapses occur only by chance, a discrete uniform distribution should be expected through the year. In the field of statistics, a discrete uniform distribution represents a probability where a finite number of values are equally likely to be observed. The most widely used example for this distribution is throwing a dice. The possible outcome values are 1, 2, 3, 4, 5, 6, and each time the dice is thrown, there is a 1/6 probability of obtaining a given number. The same statistical model can be applied to analyze the occurrence of relapses during a year. In this scenario, the probability of having a relapse should equal 1/12, or 8.3% chance of having a relapses in any given month. However, in our MS database [16], as well as in several other studies [80, 81], relapses did not follow a discrete uniform distribution: in fact there was a consistent seasonality of MS relapses, in which fewer than expected relapses occur during fall and winter, and there is a peak during spring and summer (see Fig. 1).

As a result of the regulation of its synthesis by sunlight, a significant seasonal fluctuation in vitamin D levels is observed in most geographical locations (except equatorial regions): a peak in vitamin D levels is detected in spring-summer and a nadir in autumn and winter [82]. Thus, based on the reported anti-inflammatory effects of vitamin D [83] and the fact that lower levels of vitamin D are associated with higher relapse rates [84, 85], MS relapse occurrence is predicted to peak during autumn and winter, the opposite of what is found in most locations across the globe. It can also be argued that vitamin D levels continue to be relatively low at the beginning of spring, and that the surge of relapses can be explained by the lack of vitamin D during winter and early spring, but this is insufficient to explain why relapses are higher during summer, when levels of vitamin D are already high. Although a delay of 1 or 2 months can be expected between vitamin D levels and the occurrence of a relapse, a delay in effect of 3 or more months lacks biological plausibility. Therefore, the observation of a lower occurrence of relapses in seasons characterized by lower vitamin D levels represents a "seasonal paradox": relapses should be less frequent in spring and summer when vitamin D levels are higher, yet the opposite is found in most studies [80, 81], with a few exceptions [86] (Fig. 1).

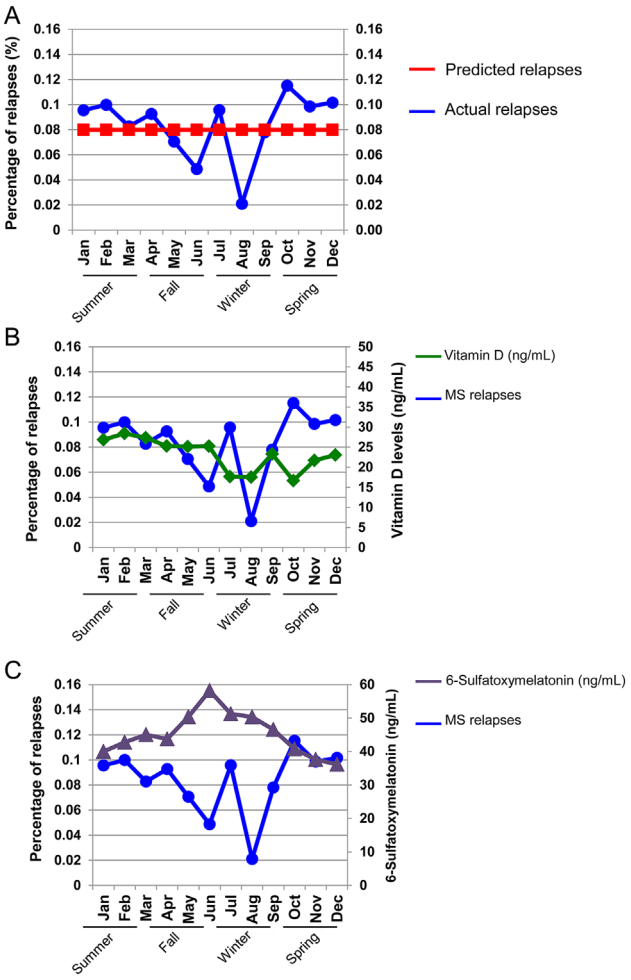

Figure 1. Seasonality of MS relapses. The distribution of observed MS relapses over the year in contrast with predicted relapses (**A**), vitamin D (**B**), and melatonin levels (**C**).

Control of CNS autoimmunity by melatonin

With this paradox in mind, we aimed to identify additional environmental factors that could account for this seasonal pattern. We focused on melatonin as an additional regulator of the immune response in MS because its levels are regulated by seasonal fluctuations in day length. Earlier evidence using melatonin as a treatment in MS animal models provided conflicting results. Constantinescu and colleagues reported initially that luzindole, a membrane melatonin receptor antagonist, suppressed Experimental Autoimmune Encephalitis (EAE) development [87]. Later, Kang and colleagues reported that melatonin was beneficial in rat EAE [88]. This beneficial effect was mediated, at least partly, by the downregulation of the cell adhesion molecule ICAM-1, thereby, reducing cell infiltrates in the CNS. However, the use of different animal models and the potent radical scavenger activity of luzindole [89] may explain the opposing effects of amelioration of EAE by treatment with melatonin or its receptor antagonist. In Table 2, we summarize the available evidence on the effects of melatonin on MS.

Recent evidence confirmed the initial findings by Kang and colleagues: melatonin ameliorates the development of EAE [16, 90]. We found that melatonin suppresses the generation of T_H17 cells via its membrane receptor MTNR1A. Upon activation, Erk1/2 becomes phosphorylated and translocates into the nucleus, where it activates the CAAT/enhancer-binding protein α (C/EBPα), a leucine zipper transcription factor involved in the regulation of cellular differentiation [91]. C/EBPα in turn represses the expression of REV-ERBα. REV-ERBα (encoded by *nr1d1*) is a component of

Table 2. Summarized evidence on melatonin and multiple sclerosis

Population	Intervention/design	Outcome/results	Reference
EAE in mice	Luzindole	Ameliorates disease	[87]
EAE in rats	Melatonin 5 mg/kg	Ameliorates disease	[88]
EAE in mice	Melatonin 5 mg/kg	Ameliorates disease	[16]
EAE in mice	Melatonin 20 mg/kg	Ameliorates disease	[90]
EAE in mice	Melatonin 200 mg/kg	Ameliorates disease	[99]
RRMS patients during exacerbation	Melatonin levels at nighttime	Decreased melatonin levels	[101]
RRMS patients during exacerbation	Melatonin levels at nighttime	Decreased melatonin levels in patients with disease duration larger than 5 years	[102]
RRMS and progressive MS	Presence of pineal calcification and brain atrophy	Increased incidence of pineal calcification in patients with brain atrophy	[103]
RRMS patients and healthy controls	Melatonin levels before and after IFN-β treatment initiation	Lower levels of melatonin in MS patients prior to treatment as compared to healthy controls. Higher levels of melatonin following IFN-β treatment	[105]
RRMS patients and healthy controls	Melatonin levels before and after Natalizumab treatment initiation	Higher levels of melatonin with Natalizumab treatment	[108]
RRMS patients and healthy controls	Melatonin levels in saliva	Decreased saliva melatonin levels in RRMS	[111]
Healthy controls	Melatonin treatment in vitro (2–500 ng/mL)	Increases secretion of IL-10 by human Tr1 cells. Decreases the production of IL-17 by T_H17 cells	[16]
One patient with PPMS	Melatonin 300 mg/day	Inhibits progression	[110]

the circadian clock that promotes T_H17 cell differentiation by limiting the expression of NFIL3, a direct inhibitor of ROR-γt transcription [92]. Because melatonin inhibits REV-ERBα expression, NFIL3 is left unopposed to block the expression of ROR-α and ROR-γt, limiting T_H17 cell differentiation and function. As a result, the differentiation of T_H17 cells is diminished and their signature cytokines, IL-17 and GM-CSF, are also affected. Of note, we found a similar effect of melatonin in human T_H17 cells. Thus, melatonin activates components of the molecular circadian clock to limit T_H17 cells in mice and humans.

In addition, we also noted that melatonin increased the levels of IL-10 producing Tr1 cells [93]. IL-27 promotes Tr1 cell differentiation through a mechanism mediated by AhR, c-Maf, and Erk1/2 hours [94–97]. We found that melatonin promotes Tr1 cell differentiation by binding to MTNR1A and activating Erk1/2 signaling, which has been previously described to induce IL-10 expression in T cells and DCs [98]. We also identified ROR-α as a mediator of the effects of melatonin in Tr1 cells. Thus, melatonin utilizes multiple pathways to boost Tr1 cell differentiation.

In agreement with our findings, Álvarez-Sánchez and colleagues reported that melatonin limits EAE development by suppressing T_H17 cells [90]. They also reported that melatonin suppresses CNS infiltration by T_H1 cells and increases $Foxp3^+$ Tregs in the CNS. Experimental differences could explain the disparities between these studies, because higher doses of melatonin were used by Álvarez-Sánchez (80 vs. 5 mg/kg in our studies). Álvarez-Sánchez and colleagues also reported that the effect of melatonin was related to the expression of CD44, a membrane receptor involved in the recruitment of cells to inflamed sites.

Finally, a recent study by Chen and colleagues also reported beneficial effects of melatonin in EAE [99]. A beneficial effect of melatonin on EAE was observed using a high dose of melatonin (200 mg/kg of body weight) during days 9, 11, and 13 after disease induction. The clinical effect was related to a decrease in the number of T_H17 cells and an increase in IL-10 production in the periphery. Melatonin also increased the secretion of IL-27 by dendritic cells, a cytokine known to induce IL-10 secretion by Tr1 cells [100]. In agreement with our findings and in contrast with those of Álvarez-Sánchez, no effect in T_H1 and $FoxP3^+$ cells was detected. Moreover, the authors detected a decrease in the number of $CD19^+$ cells in the melatonin-treated group.

An additional potential mechanism of action of melatonin in EAE was the decreased CNS infiltration associated with reduced chemokines (CCL9, CCL20) and IL-6 expression. Collectively, these data show that melatonin interferes with T_H17 cell differentiation and function while boosting regulatory IL-10-producing Tr1 cells. Further studies should clarify the role of melatonin in T_H1, $FoxP3^+$, and dendritic cells. Figure 2 summarizes the proposed mechanisms used by melatonin to limit MS pathology.

Melatonin production may be altered in MS patients

Several years ago, Sandyk and Awerbuch proposed a relationship between melatonin, pineal calcification, and MS based on several observations [101–103]. First, in a cohort of 25 patients admitted for relapses and prior the initiation of steroids, they found that melatonin levels were decreased 44% of MS patients [101]. Moreover, there was an inverse correlation between melatonin levels and disease duration. Second, in an independent cohort of 32 MS patients, the

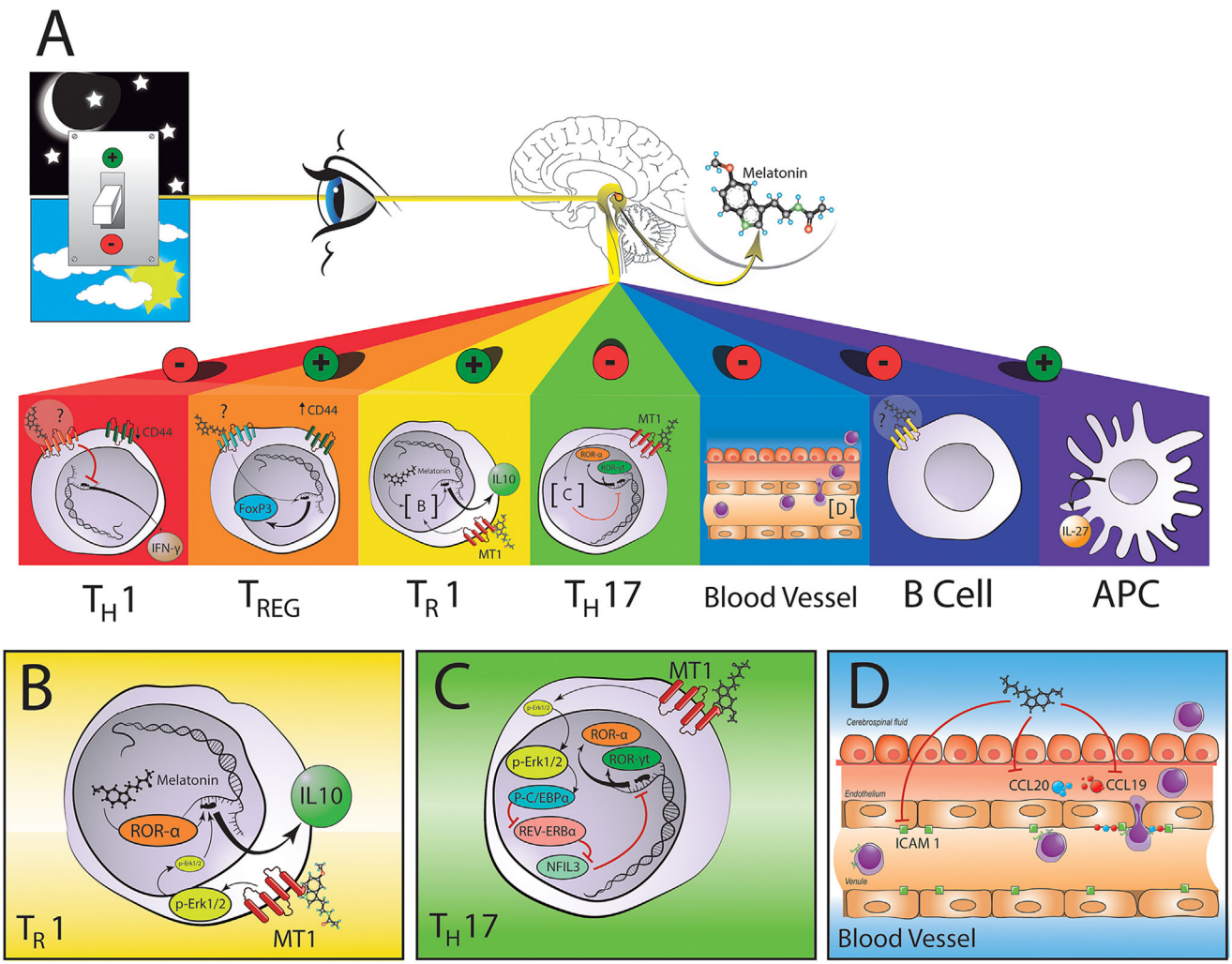

Figure 2. Mechanisms of action of melatonin in MS and EAE. **A:** The synthesis and release of melatonin is stimulated by darkness and inhibited by light. Photic information from the retina is transmitted to the pineal gland through the suprachiasmatic nucleus of the hypothalamus and the sympathetic nervous system. Melatonin affects the function of T_H1 cells by down-regulating CD44. In addition to this melatonin suppresses T_H17 cell generation via its membrane receptor MTNR1A (**B**). On the other hand, melatonin boosts Tr1 cell generation and function through the activation of MTNR1A-Erk1/2 and ROR-α, both of which promote IL-10 production (**C**). Melatonin exerts anti-inflammatory effect by potentiating the recruitment and/or function of $FoxP3^+$ Tregs to the CNS and the secretion of IL-27 by DCs. Finally, melatonin decreases the secretion of CCL20 and CCL19, as well as the expression of ICAM-1 in blood vessels associated to MS lesions (**D**).

authors confirmed their initial observations and found that patients with disease duration >5 years had lower melatonin levels than patients with shorter disease duration [102]. Third, they reported a higher incidence of pineal calcification (which is associated with lower levels of melatonin secretion) in MS patients, in particular in those with some degree of brain atrophy [103].

MS is associated with comorbidities such as hypertension, heart disease, anxiety disorder, depression, and sleep disturbances [104]. Sleep disruption is a frequent complaint of MS patients and may be related to the disease per se as well as being secondary to MS symptoms such as incontinence, pain, and spasticity. Sleep disturbance at night in turn contributes to daytime fatigue among MS patients [105]. Melamud and coworkers analyzed the relationship between melatonin levels and sleep disturbances in MS patients, and the effect of IFN-β treatment. Although no association was found between melatonin and sleep patterns, low levels of melatonin were detected in 13 MS patients before IFN-β treatment. Notably, after the initiation of therapy there was a marked increase in melatonin reaching levels comparable to those in healthy controls [105]. Of note depression, a frequent comorbidity in MS patients, is also associated with low melatonin levels but depression was not analyzed in this study [106]. Therefore, the low levels of melatonin observed in this study could potentially reflect a higher frequency of depressed individuals within the MS population. Indeed, lower levels of melatonin have been found in MS patients with depression as compared to those without depression [106].

The effects of Natalizumab on melatonin levels were also studied. Natalizumab is a humanized monoclonal antibody against the α4 subunit of the cell adhesion molecule VLA-4 expressed on the surface of lymphocytes and monocytes [107]. By preventing the interaction between VLA-4 and ICAM-1 on the brain vascular endothelium, Natalizumab prevents lymphocytes from entering the CNS and causing MS lesions. Bahamonde et al. studied the potential effects of Natalizumab on melatonin levels and oxidative stress biomarkers in a cohort of 18 MS patients followed for 56 weeks [108]. The authors detected increased daytime melatonin levels as a result of Natalizumab treatment. Of note, there is no indication of seasonal adjustment in this study. Thus, depending on the season or month in which the protocol was started, seasonal variations in melatonin levels may have influenced these results.

The relationship between melatonin and MS progression is less known. A Finnish genome study reported that polymorphisms in TPH 2 and MTNR1B genes may modulate the risk of and the accumulation of disability in progressive MS [109]. This finding implies that a dysregulation of the melatonin pathway in progressive patients may somehow facilitate the accumulation of neurological disability. However, additional validation studies are required to support these findings.

With the exception of a single-case report, there is no evidence that melatonin or its analogs are useful for the treatment of progressive MS. In 2014, López-González and colleagues reported the case of a 28-year old female diagnosed with primary progressive MS, who recovered progressively from an EDSS of 8 to an EDSS of 6 following daily melatonin administration [110]. Additional studies are needed to evaluate the appropriate pharmacological approaches to therapeutically target melatonin signaling in MS.

Is melatonin a risk factor for developing MS?

Despite all the evidence linking the immune response and melatonin in MS patients, it is unclear whether decreased melatonin levels affect the risk of developing MS. In the small case-control study mentioned above, Melamud et al. reported that MS patients had overall lower levels of 6-SM compared with healthy controls [105]. In another case-control study, melatonin levels in saliva were found to be marginally lower in MS patients as compared to healthy controls [111]. Finally, night shift work, which is associated with lower overall melatonin levels [112], was found to increase the risk of developing MS in a large case-control study [113]. These findings suggest that low melatonin levels may also be an MS risk factor. Of note, vitamin D, another environmental factor linked to MS, may also be lower in night-shift workers [114]. Thus, further research is needed to dissect the effects of vitamin D and melatonin levels on MS risk. Caution should be taken in considering potential confounders. For example, reverse causality could affect the interpretation of these studies: melatonin secretion could be impaired in MS patients as a result of CNS lesions affecting the retinohypothalamic tract and the suprachiasmatic nucleus of the hypothalamus.

Conclusions and outlook

The steady rise in the incidence of autoimmune disorders in the past 50 years cannot be explained by changes in the genetic admixture of the population. Accordingly, several environmental factors and infections have been associated with the risk or severity of autoimmune diseases. In the case of MS, smoking, vitamin D, and Epstein-Barr virus infection are remarkable for the strength and amount of evidence supporting their role in disease pathogenesis [115]. Recent studies point to melatonin as an additional environmentally related factor that impacts MS pathogenesis. There are, however, major gaps in our knowledge regarding the effects of melatonin on MS pathogenesis. First, it is currently unknown whether low melatonin levels increase the risk of developing MS, although partial support is provided by small case-control analyses and indirect evidence such as night-shift work studies. Second, vitamin D is an important environmental factor linked to MS, yet vitamin D levels are not correlated with seasonal changes in MS activity. How these findings can be reconciled is an intriguing question. In our analytical setting, although vitamin D displayed a seasonal pattern, overall levels were below national recommendations, and therefore, unlikely to have a significant immunological impact. Thus, it is possible that in patients with low levels of vitamin D, melatonin takes a leading role in preventing relapses during fall and winter. Whether this occurs in patients receiving vitamin D supplementation or with overall high endogenous levels remains to be elucidated. Third, a latitude gradient exists for MS prevalence, with higher prevalence observed in association with greater distance from the equator. This observation has been explained as reflecting latitude-linked differences in genetic and environmental factors such as vitamin D or EBV infection. Melatonin is predicted to be higher during shorter days, which is the case at locations far from the equator. Equally, a reduction in melatonin levels is expected in response to longer days in spring and summer. Thus, one can hypothesize that the further from the equator one is, the clearer the seasonal pattern of variation in MS disease activity; but this point remains to be properly analyzed. Fourth, melatonin could potentially interact with EBV by influencing virus-specific immunity, a hypothesis that also remains to be investigated. In summary, melatonin alleviates MS and other autoimmune disorders by affecting the balance between effector and regulatory cells. This finding provides a molecular mechanism to explain seasonal changes in autoimmune disease activity, and identifies melatonin signaling as a potential target for therapeutic immunomodulation.

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