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Review

Photoimmunomodulation and melatonin

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ABSTRACT

The seasons, and daily physical rhythms can have a profound effect on the physiology of the living organism, which includes immune status. The immune system can be influenced by a variety of signals and one of them is photic stimulus. Light may regulate the immunity through the neuroendocrine system leading to the most recent branch of research the "Photoimmunomodulation". Mammals perceive visible light (400–700 nm) through some specialized photoreceptors located in retina like retinal ganglion cells (RGC). This photic signal is then delivered to the visual cortex from there to the suprachiasmatic nucleus (SCN) of the hypothalamic region. Melatonin – one of the universally accepted chronobiotic molecule secreted by the pineal gland is now emerging as one of the most effective immunostimulatory compound in rodents and as oncostatic molecule at least in human. Its synthesis decreases with light activation along with norepinephrine and acetylcholine. The changes in level of melatonin may lead to alterations (stimulatory/inhibitory) in immune system. The evidences for the presence of melatonin receptor subtypes on lymphoid tissues heralded the research area about mechanism of action for melatonin. Further, melatonin receptor subtypes-MT1 and MT2 was noted on pars tuberalis, SCN and on lymphatic tissues suggesting a direct action of melatonin in modulation of immunity by photoperiod as well. The nuclear receptors (ROR, RZR etc.) of melatonin are known for its free radical scavenging actions and might be indirectly controlling the immune function.

Contents

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1. Introduction

The demand of good health is of prime importance for all living being and is essential for reproduction as well as to combat with environmental stress. For timed physiological function, almost all the vertebrate groups are dependent on environmental signals (i.e. light, temperature and humidity) [1,2] which help them to achieve high survival rate for their young ones. Among the environmental signals, the photoperiod is one of the most important environmental cues, which has a perfect timing over eons in geographically distributed zones. Further, a set of neuroendocrine mechanism is directly responsible for the timing of seasonal rhythm and ensuring that they are synchronized to the annual geophysical cycles. Till date, the majority of work on the physiological mechanism of the photoperiodic action was focused on annual reproductive regulation among the photoperiodic vertebrates. However, photoperiodic regulation of various neuroendocrine, endocrine function(s) by melatonin – a chemical component of the neuroendocrine pineal gland, released into circulation in response to light/dark cycle is also reported. For example, the neuroendocrine mechanisms that transfer day length information into melatonin secretion patterns are critical for ultimately translating environmental factors into season-specific target organ responses such as immunity [3]. Therefore, it is reasonable to suggest that animals have developed the ability to use photoperiod information to forecast recurrent conditions associated with impending changes in the seasonal environment. Other environment factors, e.g., temperature or nutrients can modulate physiological function but they are of limited value to forecast changes in season.

Adaptations in immune function present one strategy that may promote individual survival in relation to a seasonal incidence of opportunistic diseases or changes in environmental conditions. The annual change in photoperiod is the most reliable proximate cue that predicts seasonal challenges in climate, nutrition and opportunistic pathogens. Not only in mammals seasonal changes in disease prevalence and immune status were noted, but these differences are also well known among humans [4].

2. The immune system

Immune system is a truly amazing constellation of responses to attacks from outside the body. It has many facets, a number of which can change to optimize the response to these unwanted intrusions. The system is remarkably effective, most of the time with a series of dual nature, the most important of which is self/non-self recognition. The others are general/specific, natural/adaptive = innate/acquired, cell-mediated/humoral, active/passive, primary/secondary. Parts of the immune system are antigen-specific (they recognize and act against particular antigens), systemic (not confined to the initial infection site, but work throughout the body) and have memory (recognize and mount an even stronger attack to the same antigen the next time). Self/non-self recognition is achieved by having every cell display a marker based on the major histocompatibility complex. Any cell not displaying this marker is treated as non-self and attacked. The process is so effective that undigested proteins are treated as antigens.

The immune system is composed of many interdependent cell types that collectively protect the body from bacterial, parasitic, fungal, viral infections and from the growth of tumor cells. Many of these cell types have specialized functions. Often, these cells depend on the T helper subset for activation signals in the form of secretions formally known as cytokines, lymphokines or more specifically interleukins. In very simple terms, the immune system involves a variety of white blood cells that work in concert to rid the body of the presence of a foreign pathogen (antigen). The primary cell types involved in an immune response are the macrophages, the T helper/inducer cells $CD4^+$ (T4), natural killer (NK) cells, B cells and the T suppresser/cytotoxic cells $CD8^+$ (T8). The function of the macrophages is to first recognize and interact with antigen. The original antigen can also be recognized by other antigen-presenting cells such as dendritic cells or B lymphocytes. The T4 helper cells, NK cells and B cells attack and destroy the antigen. The T8 suppresser cells turn off (anergize) the immune response.

2.1. Cytokines and immune responses

Cytokines are small secreted proteins which mediate and regulate immunity, inflammation and hematopoiesis. They must be produced *de novo* in response to an immune stimulus. They generally (although not always) act over short distances and short time spans and at very low concentration. They act by binding to specific membrane receptors, which then signal the cell *via* second messengers, often tyrosine kinases to alter its behavior (gene expression). Responses to cytokines include increasing or decreasing expression of membrane proteins (including cytokine receptors), proliferation and secretion of effector molecules.

Cytokine activities are characterized using recombinant cytokines and purified cell populations *in vitro* or with knock-out mice for individual cytokine genes to characterize cytokine functions *in vivo*. Cytokines are secreted by many cell populations, but the predominant producers are helper T cells (Th) and macrophages. The most prevalent group of cytokines are various subtypes of interleukins, IL 1–23 which stimulates immune cell proliferation and differentiation for example, IL-2 stimulates proliferation of antigen-activated T and B cells; IL-4, IL-5 and IL-6 stimulates proliferation and differentiation of B cells; interferon gamma (IFN-γ) activates macrophages, IL-3, IL-7 and granulocyte macrophage colony stimulating factor (GM-CSF) stimulates hematopoiesis. Some cytokines are predominantly inhibitory for example, IL-10 and IL-13 inhibit inflammatory cytokine production by macrophages [5].

Other groups of cytokines include interferons and chemokines. Interferons (IFN-α and IFN-β) inhibit virus replication in infected cells while IFN-γ also stimulates antigen-presenting cell major histocompatibility complex (MHC) expression. Chemokines attract leukocytes to infection sites. Chemokines have conserved cysteine residues that allow them to be assigned to four groups. The groups with representative chemokines are C–C chemokines (RANTES, MCP-1, MIP-1α, and MIP-1β), C–X–C chemokines (IL-8), C chemokines (lymphotactin), and CXXXC chemokines (fractalkine) [5].

Helper T cells have two important functions; (i) to stimulate cellular immunity and inflammation and (ii) to stimulate B cells to produce antibody. Two functionally distinct subsets of T cells secrete cytokines which promote these different activities. Th1 cells produce IL-2, IFN-γ and TNF-β which activate Tc (cytotoxic T cells) and macrophages to stimulate cellular immunity and inflammation. Th1 cells also secrete IL-3 and GM-CSF to stimulate the bone marrow to produce more leukocytes. Th2 cells secrete IL-4, IL-5, IL-6, and IL-10, which stimulate antibody production by B cells. Studies proposed that modulation of cytokines are possible by circulating hormones, neurotransmitters and opioids which generally influences the immune status [6]. Further, we know that fluctuation in photoperiod daily or seasonal is equally responsible for fluctuation in immune status or function which we will deal in the next few pages.

3. Photoimmunomodulation

Light strongly influences life of all living beings on the planet through the stimulation of the visual system and the regulation of the circadian timing system [7]. The vertebrate retina contains

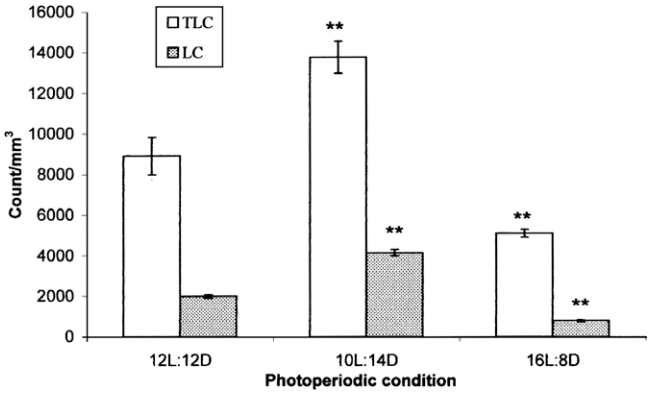

Fig. 1. Effect of different photoperiod on peripheral blood total leukocyte (TLC) and lymphocyte count (LC) of Indian palm squirrel, *Funambulus pennanti* collected at night times (22.00 h). Vertical bar represents Mean ± SEM; $N = 7$. Significance of difference from control (12L: 12D) $^{**}p \leqslant 0.01$ and $^{*}p < 0.05$.

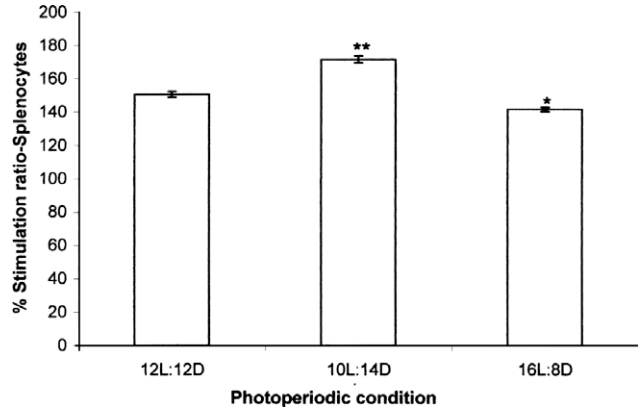

Fig. 2. Effect of different photoperiod on percent stimulation ratio (%SR) of splenocytes of Indian palm squirrel, *Funambulus pennanti*. Vertical bar represents Mean ± SEM; $N = 7$. Significance of difference from control (12L: 12D) $^{**}p < 0.01$ and $^{*}p < 0.05$.

circadian clocks that temporally regulate its physiology as well as photoreceptors responsible for the synchronization of the various physiological rhythm to environmental illumination conditions [8–10]. In the absence of formal vision, the retina may act as a sensor of the environmental illumination conditions. All wavelengths and duration of light have the potential to modify the immune response. This includes the effect on immune system due to change of seasons (circannual) and daily (circadian) light [11]. The timing, intensity and wavelength of light contribute to immune modulation. An alternative pathway for immune modulation by light is through the skin. Visible light (400–700 nm) can penetrate epidermal and dermal layers of the skin and may directly interact with circulating lymphocytes to modulate immune function. In contrast to visible light, *in vivo* exposure to UV-B (280–320 nm) and UV-A (320–400 nm) radiation can alter normal human immune function only by a skin-mediated response. Ionizing and nonionizing ultraviolet (UV) radiation (below 400 nm) have been found to suppress immune function [12] which is a skin-mediated response. Visible radiation may affect the immune system through both skin-mediated and retinohypothalamic tract mediated mechanisms. Therefore, specific areas such as the pituitary, hypothalamus and the pineal glands are stimulated to produce neurohormone [13] that could direct changes in immune function [14].

Seasonal fluctuations in light mediated changes in innate and acquired immune functions have been exclusively documented [15]. Measures of immune cell counts, lymphoid organ weights or T cell-dependent antibody responses to xenogeneic antigens are generally enhanced by short photoperiod of winter [15–17]. Moreover, laboratory experiments in which only photoperiod is manipulated indicate that exposure to short days increased mass of the spleen (splenomegaly, Ahmed, PhD dissertation 2008), as well as enhanced the numbers of total leucocytes and lymphocytes (Fig. 1). These data indicates that short days enhanced melatonin secretion, which possibly enhanced functional capabilities by lymphoid and myeloid cells, which in turn significantly increased percent stimulation ratio (%SR) of splenocytes when challenged against T-cell mitogen, Con-A (Fig. 2). Although direct measures of functions by distinct immune cell populations have not been extensively studied but tumorigenesis was reduced while basal lymphocyte proliferation or mitogen-induced splenocyte proliferation were promoted in rodents in short days [15–18]. Changes in photoperiod can have significant influences on immune function. (Ref. Ahmed, PhD dissertation 2008).

Seasonal changes in immune parameters have also been observed in non human primates [19]. There is a seasonal shift in the frequency of cells expressing Th1 cytokines (IL-2 and IFN-γ) and those expressing Th2 cytokines (IL-4) in peripheral blood mononuclear cells that were collected from rhesus monkey (*Macaca mulatta*) during the winter and summer. A related study shows that circulating numbers of white blood cells and neutrophils, and lymphocyte proliferation in response to mitogens are higher in winter than in the summer [20]. A more controlled study reported that seasonal changes in immunity of outbreed beagle dogs (*Canis familiaris*) were maintained even in open colonies. These dogs display reduced lymphocyte response to the B-cell mitogen PHA-P and the T-cell mitogen Concanavalin-A in winter relative to summer [21]. In another nonhuman animal study, seasonal fluctuation in IgM and IgE antibody forming cells, E-rosette-forming cells as well as blood and lymphoid NK-cell activity was examined in rats, rabbits and dogs [22]. Significant seasonal changes were also found in specific immune responses (e.g., IgM antibodies and K-cells), with enhancement reported during autumn and winter, compared with spring and summer. Further, less dramatic changes were reported for IgE or E-rosette-forming cells in those small rodents [22].

Alterations in photoperiod have profound effects on levels of the hormones testosterone, estradiol and cortisol, all of which have been found to alter immune cell function [23]. It is known that prolonged changes in the natural photoperiod adversely affect the immune response of tropical rodents but rapidly resume normal function after returning to a normal photoperiod. It has been found that changes in the photoperiodic regime of squirrels produce detectable chronic stress under constant light/dark condition in the form of elevated cortisol [24].

Immune cell function is generally enhanced by exposure to short days is based on the hypothesis that in seasonal breeder distinct immune cell functions are influenced by ambient photoperiod [2]. The Siberian hamster and Indian palm squirrel were chosen as the animal model because the neuroendocrine mechanism that mediates photoperiod control of immunity has been studied extensively in those two species. Findings from these studies indicate that profound but selective effects on immune functions are associated with the prevailing photoperiod [25,26]. Photoperiod and melatonin treatment have been shown to affect reproductive competence, sex hormone secretion together with selected parameters of immune function [27]. This suggests that the hypothalamo-pituitary–gonadal axis may be involved in the mediation of melatonin action on the immune system [28].

3.1. Light and local immune responses

The mechanisms responsible for local immunosuppression in the UV-B-irradiated skin are well documented [29,30]. Keratinocyte-derived cytokines are generally considered to be the initiators of the local effects of UV-B resulting in the production of a plethora of cytokines within the epidermis [29] and crucial for the observed local immunological unresponsiveness. Based on their cytokine secretion patterns, the $CD4^+$ Th cells can be divided into at least two effector populations: Th1 and Th2 cells. The Th1 population produces IL-2, lymphotoxin and IFN-γ, whereas the Th2 cells produce IL-4, IL-5, IL-6 and IL-10 [31]. It is generally assumed that especially Th1-mediated responses are sensitive to UV-B exposure [32–34], but the mechanisms by which UV-B affects Th1 responses and the consequences for Th2-mediated responses are not yet clear.

Regulatory T cells (Tr) play an important role in the regulation and suppression of immune responses, respectively [35–37]. Several studies further characterize UV-induced suppressor T cells; designated as UV-induced regulatory T cells [38–41] most of these belong to the CD4 type [42]. There is recent evidence that they also express CD25 [43] and CTLA-4 [44]. In addition, they secrete IL-10 upon hapten-specific stimulation [38–41]. The observation that UV-mediated tolerance and transfer of suppression can be inhibited by neutralizing anti-IL-10 antibodies suggests that the release of IL-10 by UV-induced Tr plays an important role in photoimmunosuppression [41]. Although the UV-induced suppression of contact hypersensitivity (CHS) and delayed type hypersensitivity (DTH) is clearly mediated via T cells while UV-induced suppression of tumor immunity appears to be mediated via NKT cells [45].This is an area where the role of melatonin in UV mediated local immune response is completely lacking. The wavelengths of light transmitted through different layers of skin do not vary dramatically from lower mammals to primates. The longer the wavelength of light is, the deeper will be the penetration in skin. The shortest wavelengths of UV light elicit the strongest immune response [46] whereas the skin-mediated visible light response is weak but detectable. It is therefore important when reporting skin mediated neuroendocrine immune findings, to control the intensity, timing and wavelength of ambient light.

3.2. Photoperiod and seasonal immunity

Seasonal biological function commonly indicates a role for photoperiod as a mediator in physiological adaptations to changes in the environment. Photoperiod control of seasonal reproduction is a significant example. Eye–brain mechanisms are both species specific and age dependent and are determined by the wavelengths of light transmitted through the eye and reaching the retina that can then be transmitted to the brain. The wavelengths of light that might induce an eye–brain-mediated immune response depend upon the transmission properties of the eye of the particular species. Both UV and visible light may induce an eye–brain-mediated neuroendocrine response. This photic signal is transferred to the retina and sent to the visual cortex for vision and through alternative pathways to the hypothalamic, pineal and limbic structures (Fig. 3). Vertebrate retinal ganglion cells (RGCs) are responsible for sending photic information to the brain that synchronizes endogenous clocks to the environmental lighting conditions [7,8,10]. A subset of RGCs in mammals participates in an independent circuitry that regulates a number of non-image forming tasks, i.e. light entrainment of activity rhythms, pupillary light responses and melatonin suppression by light, sleep, and masking [8]. RGCs that expressed photo pigment melanopsin [47] were shown to be intrinsically photosensitive [48] and proposed to act as circadian photoreceptors. Till date, the nature of the biochemical events operating in the circadian phototransduction of vertebrates is still uncertain.

Seasonal rhythms in immune response have been documented in many species [2,16]. T cell immunity was found to be depressed in most species in the winter even when natural light sources (photoperiod) are kept constant. There is a direct correlation

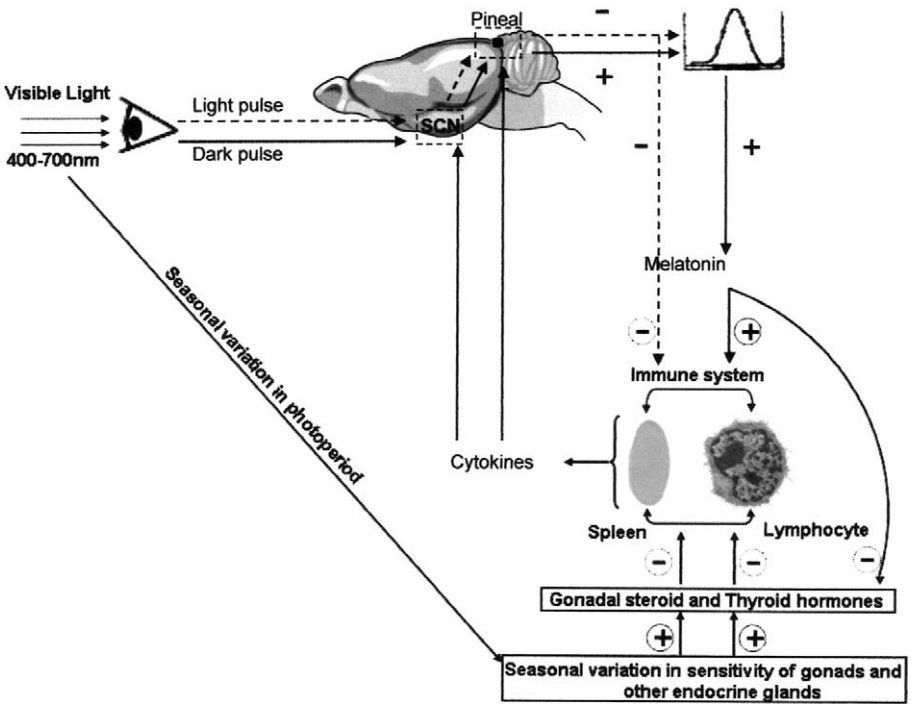

Fig. 3. Hypothetical diagram showing light regulation of neuroendocrine immune system in rodent. Long day length reduces circulating night time melatonin which in turn increases gonadal activity and reduces immune status.

between changes in immune response during visible light treatment and the seasons in humans [11]. Reports are available suggesting that immune cell numbers and immunoglobulin concentrations vary with respect to the season or day length [25,26] raising the possibility that photoperiod may also influence functional capabilities of immune cells. Correlation between the changes in functions of certain immune cells associated with exposure to short days has been reported (Fig. 1). Short days selectively enhance natural killer cell activity and basal spontaneous proliferations but contrast, phagocytic and oxidative burst activity, particularly by granulocytes gets reduced in hamsters under short vs. long days [27].

Being a photoperiodic neuroendocrine transducer, the pineal gland also receives input from the peripheral immune system suggesting bi-directional relationship [49]. In our animal model, the tropical Indian palm squirrels *Funambulus pennanti*, we observed increase in immunity in squirrels under short days suggesting a strong influence of endogenous melatonin in maintenance of immune status in this seasonal breeder during winter [25]. We further observed that a certain part of the year they are susceptible to more infections and season bound diseases particularly during the transitional reproductive phases [18,25]. Indian palm squirrels during reproductively active phase in summer months are healthy even though the peripheral level of the so-called immunoenhancing hormone, melatonin is low. Actually the favorable environmental conditions of long photoperiod, enough food along with high peripheral gonadal steroid(s) might be responsible for their good health [50]. On the other hand, during reproductively inactive phase when the environmental conditions are not favorable (less food, low temperature and short photoperiod etc.) to the squirrels, the circulating high melatonin level (due to winter month or short photoperiod) might have enhanced the immune function to keep them healthy to surpass winter [15,18,25].

3.3. Photoperiod and daily variation in immunity

Immune function like other common physiological and behavioral process undergoes daily variation. Most mammals respond to seasonal changes of day length photoperiod with altered physiology and behavior. Photoperiodic information from the environment is conveyed to the organism by a circadian rhythm of melatonin production by the pineal gland [50–53] that inhibits reproductive function or enhance immunity in many seasonally breeding rodents [3,50]. This inhibition in reproduction causes reduction in several hormones mostly steroids, gonadotrophins, GnRH and prolactin. Importantly, both estrogen and androgen affects immune function. Testosterone, primary androgenic hormone secreted by males of most species, typically suppresses immunity [17,54]. Thus, enhanced immune function in short days or treated with melatonin may be caused by reducing circulating testosterone concentration. In contrast estrogen in females generally enhance immune function [26,54]. Both males and female rodents showed enhancement in cellular immunity when exposed to short day length [55]. All laboratory studies of photoperiodic changes in immune parameters of mammals demonstrated enhanced immune function in short winter-like photoperiod. For example, deer mice significantly increased lymphocyte, neutrophil and white blood cell counts in short photoperiods [56]. Increased lymphoproliferative activity and changes in spleen morphology occur in hamsters under short day length [16]. Additionally, deer mice under short days showed enhancement in both cell-mediated as well as humoral immunity. Recently, the interactive effects of photoperiod and ambient temperature on immunity were examined in deer mice [57,58]. In another experiment when animals were kept briefly under long or short days and in mild or low ambient temperatures, total serum immunoglobulin (Ig) G concentration increased in short day at mild temperature compared to long-day mice. Taken together, these results it appeared that there is not much discrepancy noted between field studies demonstrating reduced immune function in winter compared to summer and laboratory studies demonstrating enhanced immune function in short winter-like photoperiods compared to long summer-like day lengths. The net effect of elevated immune function in short days appears to be to counteract the suppressive effects of environmental stressors such as low ambient temperature on immune function [58]. Although photoperiod is the primary cue for seasonally breeding animal species, many other environmental factors, including temperature, humidity, rainfall and food availability varies on a seasonal basis. Some of these factors may be perceived as stressful [59] and can affect immune status since, photoperiod regulates melatonin synthesis and thereby immune status. This part is being discussed in detail under separate heading.

Daily rhythm of photoperiod has a profound effect on immune responsiveness in humans [60–63]. The immune response to various antigen presentation differs both quantitatively and qualitatively depending upon the time of exposure to light. In mammals the proliferation and circulation of T, B or NK lymphocytes in the peripheral blood differs throughout the day. T lymphocyte response to antigen and proliferation of those cells is most efficient in the morning on the other hand B cells have maximum antigen response, proliferation and circulation in the evening. Further, the enhanced expression of IL-2 receptors and proliferation of NK cells appear in the early afternoon. While the IL-2RmRNA synthesis for T cells peaks at 1 am for B cells at 10 am and for NK cells at 7 am.

4. Melatonin and immunity

In recent years much attention has been devoted to the possible interaction between melatonin and the immune system [64–67]. Melatonin has significant immunomodulatory roles in immunocompromised states where endogenous melatonin was eliminated both functionally and pharmacologically [66,67]. In 1986, Maestroni et al., first showed that inhibition of melatonin synthesis causes inhibition of cellular and humoral responses in mice [68]. Mice kept under constant light or receiving injections of β-adrenergic blockers (propranolol) to inhibit melatonin synthesis exhibited an inability to mount a primary antibody response to sheep red blood cells (SRBC), a decreased cellularity in thymus and spleen and a depressed autologous mixed lymphocyte reaction. All those were reversed by melatonin administration when given in the late afternoon [68] or in the presence of T-dependent antigenic stimulation. In these experiments melatonin was ineffective *in vitro*. Maestroni and co-workers (1987) concluded that, it might have exerted immunostimulating effect through other neuroendocrine mechanisms in antigen-activated cells [69]. Later on handful of evidences came up to show that melatonin influences lymphocyte *in vitro* [70,71]. Hamsters exposed to short photoperiods had increased spleen weight and number of splenic lymphocytes and macrophages [72].

4.1. Melatonin and cytokine production

Melatonin has been proposed to regulate the immune system by affecting cytokine production in immunocompetent cells [73,74]. Melatonin enhances the production of IL-2, IFN-γ and IL-6 by cultured human mononuclear cells [75]. Melatonin, by activating monocytes [76], increases the production of IL-1, IL-6, TNF-α and reactive oxygen species. Melatonin also increases IL-12 production by monocytes [76]. Repeated stimulation of T helper (Th) cells in the presence of IL-12 causes Th cells to differentiate

into Th1 cells which produce IL-2 and IFN-γ and are particularly effective in enhancing immune responses that involve macrophages and other phagocytes. Melatonin augments IFN-γ production by Th1 cells [74]. The enhancement of NK-cell activity by melatonin is attributed to the increased production of IL-2 and IL-12 [74,77].

Human lymphocytes themselves play an important role in stimulating IL-2 production in an autocrine or paracrine fashion [78]. After melatonin treatment up-regulation of gene expression for TGF-β, M-CSF, TNF-α and stem cell factor (CSF) in peritoneal exudate cells and the level of gene expression of IL-1β, M-CSF, TNF-α, IFN-γ and SCF were reported in splenocytes [79]. Melatonin's immunoenhancing effect also depends upon its ability to enhance the production of cytokines as well as its anti-apoptotic and antioxidant action [80–82]. As a functional impairment of macrophages and granulocytes (as shown by the diminished intracellular phagocytotic activity, degranulation and decrease in chemotactic activity) has been reported in the elderly [83,84] where a parallel decrease in melatonin production occurred [85–87]. It may not be unreasonable to speculate that immunosenescence can be partly attributed to a decreased production of melatonin. To restore the defective phagocytic function the use of melatonin adjuvants with immunizations and nutritional supplements has been proposed [88].

Melatonin has been shown to aggravate Th1 dependent inflammatory response in animal models of multiple sclerosis [89] and rheumatoid arthritis [90]. Importantly, many studies have suggested that melatonin may act as an anti-inflammatory compound. This action of melatonin is at least partly due to induction of Th2 lymphocytes that produce IL-4, thereby inhibiting the function of Th1 cells [91]. Indeed, melatonin has been shown to be protective in septic shock [92], an animal model of ulcerative colitis [93] and experimental pancreatitis [94,95]. Recently, melatonin has been found to reduce NF-kB (nuclear factor-kappa B) binding to DNA, probably by preventing its translocation to the nucleus [96]. This in turn reduced the production of pro-inflammatory cytokines and chemokines. Additionally, because melatonin has been shown to reduce adhesion of leukocytes to endothelium as well as transendothelial migration, it might have also suppressed the expression of NF-kB- regulated adhesion molecules [97]. Finally, melatonin has been shown to reduce recruitment of neutrophils to the site of inflammation [98,99].

Some reports also suggest role of melatonin in immunopathological conditions such as acute and chronic inflammation [100,101]. 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-γ, IL-1β, IL-6, IL-12, IFN-γ and NO [102]. Most studies relating to melatonin and endotoxin-induced processes observed that administration of melatonin improved the survival of mice and rats from a lethal dose of LPS with survival rate higher than 80% [103–105]. In this context, melatonin has been shown to prevent endotoxin-induced circulatory failure in rats through an inhibition of TNF-α levels [105] and to reduce postshock levels of IL-6 in male C3H/HeN mice [106].

4.2. Melatonin and immune function in humans

Melatonin also found to play a potential role in physiological function of human beings. The apparent clock-setting property of melatonin has led to the suggestion that it is a "chronobiotic" substance that alters and potentially normalizes biological rhythms and adjusts the timing of other critical processes and biomolecules (hormones, neurotransmitters, etc.) that in turn exert numerous peripheral actions [107].

Immunomodulatory effects of melatonin were also observed recently in healthy subjects and patients with bronchial asthma [108]. Melatonin increased production of interleukin IL-1, IL-6 and TNF-α indicating the possibility of an adverse effect of exogenous melatonin in patients with asthma. On the other hand, in a model of adjuvant-induced arthritis, both prophylactic and therapeutic melatonin administrations inhibited the inflammatory response [109]. This inhibition was accompanied by enhanced thymocyte proliferation and IL-2 production by melatonin. In another animal study, melatonin was shown to possess both cellular and humoral immunoenhancing effects and immune responses were augmented even in the absence of previous immunosuppression [110]. Melatonin-receptor immunoreactivity has also been detected in the human eye [111], the physiologic function of which remains unclear. A key finding-albeit in young adult humans – with respect to the interplay of melatonin and the immune system, was the observation that the nocturnal rise of blood melatonin in humans correlated with the increase of thymic production of peptides like thymosin-1 alpha and thymulin [112].

Pinealectomy stimulates and/or melatonin inhibits the growth and sometimes the metastasis of experimental cancers of the lung, liver, ovary, pituitary, and prostate as well as melanoma and leukemia [113]. Clinical evidence suggests a role for melatonin in the prevention and even the treatment of breast cancer [114]. For example, the circadian amplitude of melatonin was reduced by more than 50% in patients with breast cancer vs. patients with non-malignant breast disease [115] and high melatonin levels have been found in morning urine samples of breast cancer patients [116] suggesting circadian. In another study, melatonin 10–50 mg daily at 8 pm potentiated IL-2 immunotherapy of pulmonary metastases [117]. Melatonin antagonizes several effects of exogenous corticoids inducing immune depression [118], hypercatabolism, thymic involution and adrenal suppression [119]. These findings have led to the suggestion that melatonin might work as an antiadrenocortical or antistress factor [119]. The melatonin/corticoid relationship is significant because chronic hypercortisolemia has been linked to several aspects of aging and age-associated phenomena including glucose intolerance, atherogenesis, impaired immune function and cancer [120]. In addition to high absolute levels of corticoids, disorganization of the normal rhythm of corticoid release is also pathogenic. Corticoids are normally high in the early morning and daytime and low at night. Properly timed exogenous melatonin may entrain or reorganize this critical endocrine rhythm resulting in long-term systemic benefit. Indeed, the immune-enhancing and anticorticoid effects of melatonin or putative mediators of melatonin action appear to depend on nocturnal administration [118,121]. This may represent an integral immune-recovery mechanism by which melatonin acts as a kind of buffer against the harmful effects of stress on immune homeostasis [118].

Beta-adrenoreceptor blockers which depress melatonin secretion, exert immunosuppressive effects, but only when given in the evening [122,117]. This is when blood melatonin (and the immunoenhancing effect of melatonin) is highest. Exogenous melatonin reverses beta-blocker-induced immunosuppression and enhances immune parameters in animals. A preliminary report of patients with AIDS who took melatonin 20 mg daily in the evening revealed uneven but generally beneficial effects on immune parameters [123]. It has been recommended that the dose be timed not only periodically within each day (at night only) but also periodically within the month, with treatment periods of 3–4 weeks followed by a week-long "washout" period [117]. Role of melatonin on immune regulation in human is getting added up day by day. But, whether in human photoperiod regulates immunity *via* melatonin secretion as evidenced in small mammals needs to be clarified.

4.3. Melatonin receptors

Two mammalian subtypes of G protein coupled melatonin receptors, MT1 (Mel1a) and MT2 (Mel1b), have been cloned and characterized [124–127]. The human MT2 receptor has a lower affinity (Kd = 160 pmol/l) for ^{125}I-melatonin as compared to the human MT1 receptor (Kd = 20–40 pmol/l), but the binding characteristics of the two are generally similar, e.g., both are of high affinity and the agonist binding is guanosine triphosphate (GTP)-sensitive [127]. A third and low affinity receptor which has been discovered recently and named as "MT3" was later characterized as the enzyme quinone reductase-2 [128]. This enzyme belongs to a group of reductases that participate in the protection against oxidative stress by preventing electron transfer reactions of quinones.

Melatonin is also a ligand for a retinoid related orphan nuclear hormone receptor (RZR/ROR) [129]. These nuclear receptors belong to the RZR/ROR orphan receptor subfamily, which includes three subtypes (α, β, γ) and four splicing variants of the subtype [130]. RORα1 and RORα2 seem to be involved in some aspects of immune modulation and RZRβ is expressed in the central nervous system including the pineal gland [131,132]. Moreover, RORα was assumed to mediate up-regulations of antioxidant enzymes [131]. Still, the full spectrum and physiological meaning of these receptors remains to be clarified.

In addition, melatonin interacts with intracellular proteins such as calmodulin [133], calreticulin [134] or tubulin [135,136] and antagonizes the binding of Ca^{2+} to calmodulin [137]. The affinity of calmodulin to melatonin is sufficient for mediating effects at elevated physiological concentrations, especially those attained in tissues [137–139]. Melatonin binding results in inhibitions of CaM kinase II [139] and of neuronal nitric oxide synthase [140]. Moreover, melatonin causes PKCa-dependent phosphorylation of calmodulin [141], presumably by signaling mechanisms described in the preceding section, but this effect is important so far as it perpetuates CaM-dependent inhibitions. Interacting calmodulin and kinase effects are relevant to rearrangements of the cytoskeleton [137] which represent some of the earliest effects described for melatonin, including ciliates and plants [142]. An additional facet of melatonin/Ca^{2+} interactions is the binding to calreticulin and perhaps to two nuclear proteins, one of which had high homology to calreticulin, whereas the other was structurally different [134]. These interactions are most likely related to some of the physiological effects of melatonin but critical data regarding this point have yet to be obtained.

4.4. Distribution of melatonin receptors

Although melatonin receptors have been localized in number of lymphoid tissues in both nucleus as well as in cytoplasm. 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 rodents [143–146], mouse peritoneal macrophages [147] and in human lymphocytes [148]. 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), cyclic GMP (cGMP) and diacylglycerol (DAG) production [148]. Poon and Pang [143] had observed binding sites localized in the nuclear fraction (65.5%) of guinea pig spleens. In the mouse thymus, Liu et al., [149] showed that the subcellular distribution of binding sites was mostly located in the nuclear fraction. Purified cell nuclei of spleen and thymus of rats had melatonin nuclear binding sites, with Kd values of 0.068 nM and 0.102 nM, respectively [150];

(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 MT1-melatonin receptor mRNA in T and B subsets of lymphocytes from rat thymus and spleen was shown in 1997 [151]. Later, Garcia-Maurino et al., [152] reported that Jurkat cells expressed the mRNAs for the nuclear receptors RZRα, RORα1, RORα2 and for the membrane receptor MT1 whereas U937 cells, a monocytic cell line, expressed MT1 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 MT1 receptor [153]. MT1 receptor mRNA is present in the rodent SCN, paraventricular thalamus and in the pars tuberalis (PT) [126]. This correlation between the distribution of receptor binding and the distribution of MT1 receptor mRNA, in sites thought to be important for circadian immune responses to melatonin [126]. At the time however, the existence of the MT2 receptor subtype was not known subsequent studies have addressed the relative role of the MT1 and MT2 receptor subtypes in mediating responses to the hormone. Subsequent studies confirmed the MT1 mRNA presence in PBMCs cell populations as well as the presence of RZRα, RORα1 and RORα2 mRNAs [154]. Recently, the presence of MT1and RORα mRNA and protein has also been detected in both the thymus and spleen of mice while, MT2 receptor mRNA was also detected but only in the thymus [153] and

(c) the development of several specific melatonin membrane and nuclear receptor agonists and antagonists [155] 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 [147]. 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 [156] and MT1 -/- mice [157], suggesting a direct interaction of MT2 receptor in the process. 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

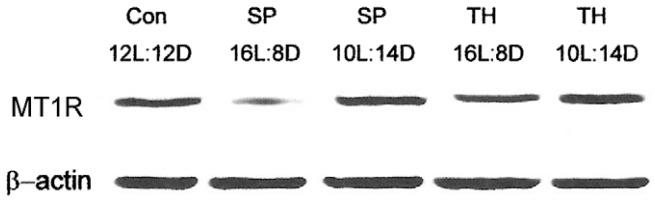

Fig. 4. Western blot analysis of MT1 receptor expression in spleen and thymus of *F. pennanti* under different photoperiodic regimes. β-actin expression was used as loading control. Con – control; SP – spleen; TH – thymus.

specific nuclear melatonin receptor agonist, mimics the stimulatory effect of melatonin on IL-2 and IL-6 production by human PBMCs, while the membrane MT1 receptor agonist S 20098 failed to activate the production of either cytokine [151]. 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. Further, Garcia-Maurino et al., [151] observed a synergistic effect of S 20098 and CGP 52608 on IL-6 production by human PBMCs. Later, Carrillo-Vico et al., [153] 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. Many more stones are needed to turn to understand exactly how melatonin utilizes light signal to modulate immunity *via* its receptor subtypes.

4.5. Mechanism of action of melatonin in immunomodulation

Four different mechanisms of action have been suggested for melatonin: (a) binding to membrane receptors [124]; (b) binding to nuclear receptors [158]; (c) interaction of melatonin with calmodulin [138,159,160] and (d) antioxidant activity [161]. By using melatonin membrane (S 20098) [162] and nuclear (CGP 52608) [158] receptor agonists, the involvement of a nuclear mechanism in the melatonin effects on IL-2 and IL-6 production by human PBMCs and IL-6 production by cultured monocytes was reported [151].

In the immune system, melatonin acts on specific membrane receptors (MT1 & MT2) expressed on immunocompetent cells with MT2 receptors playing apparently the major role in mice only [163,164] but further study needs to elucidate the exact role of MT1 & MT2 receptor in immunomodulation, especially in seasonal breeders. There is evidence of melatonin receptors mainly in human circulating $CD4^+$ T helper lymphocytes with few in $CD8^+$ cytolytic cells and none in B lymphocytes. Such a predominant effect on $CD4^+$ cells is supported by the observations on melatonin efficacy to augment $CD4^+$ cells in lymph nodes [165]. However, expression of MT1 receptor was found in rat and squirrel thymus and spleen (Fig. 4). Melatonin receptor mRNA being expressed in all the thymic lymphocyte subpopulations ($CD4^+$, $CD8^+$, doubled positive, doubled negative and B cells), indicating possible effects of melatonin on all these cells [166]. Nuclear melatonin receptors may also mediate immunomodulation since, drugs that bind to retinoid Z receptor/retinoid acid receptor-related orphan receptors (RZR/ROR) are active in experimental models of autoimmune diseases [167]. Nuclear receptors have been described in lymphocytes [168]. Melatonin is also a potent antioxidant, acting itself rather than through specific binding sites [169]. In addition, melatonin could affect centrally the release of hormone in the hypothalamic-hypophyseal unit [170] as well as the activity of autonomic pathways to the lymphoid organs [171].

5. Conclusion

Light does modulate the immune system through eye–brain–pineal–melatonin pathway and also influences through skin. Longer the wavelength, greater would be the penetration of light through ocular and dermal tissues. The potential to suppress or activate the immune response depends mostly on the wavelength of light which also induces specific changes in the production of neuroendocrine hormone–melatonin, which in turn may directly modulate the immune responses *via* its membrane receptors (MT1 & MT2) present on lymphoid organs. Nuclear melatonin receptors may also mediate immunomodulation since, certain drugs binds to the retinoid related orphan receptors.

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