Reprod. Nutr. Dev. 39 (1999) 383–398

Review article

Melatonin mediates seasonal adjustments in immune function

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(Received 20 January 1999; accepted 16 April 1999)

Abstract — In addition to seasonal changes in reproductive function, seasonal changes in immune function are mediated by the pineal hormone, melatonin. Melatonin affects immune function both indirectly, acting through other hormones, and directly by acting on components of the immune system. Melatonin also affects tumorigenesis and tumor development. We hypothesize that many of the indirect effects of melatonin on immune function are mediated through glucocorticoids, and appear to be part of an integrated series of adaptations to manage energy. Direct effects of melatonin on immune function appear to be mediated by melatonin receptors on lymphatic tissue or on immune cells in circulation. Winter is energetically demanding and stressful; thermoregulatory demands typically increase when food availability decreases. Individuals would enjoy a survival advantage if seasonally recurring stressors could be anticipated and countered by bolstering immune function. To summarize, melatonin may be part of an integrative system to coordinate reproductive, immunologic and other physiological processes to cope successfully with energetic stressors during winter. © Inra/Elsevier, Paris

melatonin / seasonality / thermoregulation / energetics / metabolism / photoperiod

Résumé — La mélatonine traduit les variations saisonnières de fonction immunitaire. Comme pour les variations saisonnières de reproduction, l'hormone pinéale, mélatonine, traduit les changements saisonniers de fonction immunitaire. La mélatonine modifie la fonction immunitaire à la fois indirectement, par l'intermédiaire d'autres hormones, et directement en agissant sur des éléments du système immunitaire. La mélatonine influence également la genèse et le développement des tumeurs. Nous émettons l'hypothèse que la plupart des effets indirects de la mélatonine sur la fonction immunitaire impliquent les glucocorticoïdes, et semblent faire partie d'un ensemble de mécanismes adaptatifs intégrés destinés à gérer l'énergie. Les effets directs de la mélatonine sur la fonction immunitaire semblent impliquer des récepteurs à la mélatonine sur le tissu lymphatique ou sur les cellules immunitaires de la circulation sanguine. L'hiver est coûteux sur le plan énergétique et il est source

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de stress ; les exigences de thermorégulation augmentent quand les disponibilités alimentaires diminuent. Les individus auraient plus de chance de survivre si les sources de stress qui apparaissent de manière saisonnière pouvaient être prévues et contrebalancées par une fonction immunitaire renforcée. En résumé, la mélatonine peut faire partie d'un système intégré qui coordonne les fonctions reproductive et immunitaire et d'autres fonctions physiologiques afin de supporter avec succès les sources de stress énergétique pendant l'hiver. © Inra/Elsevier, Paris

mélatonine / saisonnalité / thermorégulation / énergie / métabolisme / photopériode

1. INTRODUCTION

Melatonin, the primary hormone secreted from the pineal gland, is an indole-amine that is found throughout the animal kingdom [54, 60, 127, 133]. Melatonin encodes day length (photoperiod) information, and appears to be the primary hormone orchestrating the seasonal changes in reproductive function observed among many vertebrate animals living in mid to high latitude habitats [8, 57]. Because it regulates the onset of puberty, as well as the seasonal pattern of reproductive function and quiescence, melatonin is usually considered to be a reproductive hormone [8, 127]. In addition to reproduction, melatonin also affects a wide range of seemingly unrelated physiological, morphological and behavioral processes. In this review, the role of melatonin in the integration of immune function within the context of other energy-conserving seasonal adaptations will be emphasized [86]. Many seasonal adaptations, including suppressed breeding, increased thermoregulatory capacities, curtailed growth and enhanced immune function, have evolved to help animals cope with the annual changes in environmental energy demands. For example, melatonin appears to affect body mass regulation, gut efficiency, metabolic rate, pelage development and nonshivering thermogenesis (NST) [66, 67, 86, 132, 133]. Melatonin also affects several energy-saving behaviors including nest-building, torpor and food intake [8, 87, 124]. The inhibitory effects of melatonin on reproductive function also represent an energy-saving adaptation – animals have been selected to forego breeding when reproductive success is unlikely. Presumably, reproductive regression reflects the energetic incompatibility of breeding (e.g. mating, lactating, resource defense) and thermoregulatory activities during winter. However, the existence of winter breeding in virtually every population of small mammals studied indicates that this energetic incompatibility can be resolved [107, 111].

Photoperiodic information is used to initiate or terminate specific seasonal adaptations, including reproduction and growth, in order to maintain a positive energy balance (reviewed in [7, 65, 67, 133]). The annual cycle of changing photoperiod is a very precise temporal cue for determining the time of year. Ambient photoperiodic information is transduced by the pineal gland into a melatonin signal; peak melatonin concentrations occur during the dark and basal concentrations occur during the light portion of the day. The secretory pattern of melatonin allows individuals to ascertain the time of year and thus anticipate predictable seasonal environmental changes (reviewed in [7, 127]).

Although maintenance of a positive energy balance is critical for survival and reproductive success (reviewed in [27, 109]), other threats to survival must also be met in order for individuals to increase their fitness [113]. They must avoid predators and potentially dangerous interactions with conspecific competitors, as well as avoid succumbing to disease. Melatonin is critical in mediating the seasonal pelage color changes that are necessary for cryptic behaviors required by successful individuals of both

prey and predator species [46, 59]. Melatonin also mediates the seasonal decrease of steroid hormones; low blood androgen concentrations reduce agonistic behaviors [108]. For example, communal huddling, an energy-saving behavior, occurs much more frequently among individuals of many rodent species during the winter as compared to summer (e.g. [86, 90]); these rodents are highly aggressive and territorial during the spring and summer when sex steroid concentrations are high [109]. Taken together, melatonin appears to coordinate many seasonal and daily changes in energy use and energy conservation [70]. Energy must be conserved during winter because of the energetic bottleneck that is formed by the increased energetic needs of winter thermoregulation when food availability is limited. Indeed, coping with this energetic shortage may result in a full stress response during which muscle is catabolized, growth and other maintenance functions are curtailed, and immune function is compromised [134].

Our working hypothesis is that long-night patterns of melatonin enhance immune function in advance of stress-induced immunosuppression; thus, in nature short photoperiods provide physiological anticipation of challenging winter conditions. Energetically challenging conditions such as low temperature or limited food availability could compromise immune function in the wild [114]. The long-night pattern of melatonin secretion appears to direct energy from growth and reproduction to thermogenesis and immune functions. Laboratory studies of seasonal changes in mammalian immune function consistently report that immune function is enhanced in short day lengths (reviewed in [111, 114]). Prolonged melatonin treatment mimics short days, and also enhances certain features of immune function in rodents. Importantly, not all aspects of immunity are enhanced by short-day exposure (e.g. [32]). No data have been reported that determine if certain components of immunity are most 'cost-effective' in terms of energetics or might be more important for survival during one season of the year as compared to another. These issues deserve further study. The effects of short days and melatonin treatment on immune function are reviewed below.

2. PHOTOPERIOD CONTROL OF REPRODUCTION AND IMMUNE FUNCTION

Many of the first indications of an effect of photoperiod on immune function came from Russell Reiter's group which established photoperiodic and melatonin effects on spleen size, as well as on chemically induced mammary tumor development. Short day lengths increase splenic weight of deer mice (*Peromyscus maniculatus*) [144] and Syrian hamsters (*Mesocricetus auratus*) [25]. Splenic masses, total splenic lymphocyte numbers and macrophage counts were significantly higher in hamsters exposed to short photoperiod as compared to animals exposed to long photoperiod [25, 142]. Photoperiod did not affect thymic weight or antibody production in hamsters [24]; however, immunization procedures or assessment of splenic immunoglobulin production might have masked subtle effects of photoperiod in this study. Because these data were evaluated from the perspective of seasonal breeding where large gonads are typically functional and small gonads are typically nonfunctional, it has been assumed that larger spleens are indicative of 'better' function than smaller spleens. However, it is also possible that hypertrophied spleens reflect increased splenic activity because of disease or compromised immune function [114]. However, a wide variety of direct tests of immune function have established that immune function is often enhanced by housing in short days or chronic melatonin treatment.

To what extent does immune function correlate with reproductive responsiveness to photoperiod? Among individual short-day deer mice that showed elevated splenic

lymphocyte proliferation, there was no relationship between the degree of testicular regression and the amount of splenocyte proliferation [40]. These results suggest that photoperiodic responsiveness of immune function is not linked to reproductive responsiveness to day length. The effects of photoperiod and melatonin treatment on reproductive and immune function were assessed in two subspecies of *Peromyscus maniculatus* from different latitudes of origin [44]. Short-day *P. m. bairdii* (latitude = 42° 51' N) displayed reproductive regression and elevated splenocyte proliferation in response to the T-cell mitogen Concanavalin A (Con A), as compared to long-day mice. In contrast, *P. m. luteus* (latitude = 30° 37' N) did not undergo reproductive regression in short days; individuals of this subspecies also failed to exhibit any increase in lymphocyte proliferation to Con A in short days. Other individuals of both subspecies were implanted with empty capsules or capsules that contained melatonin. Individual *P. m. bairdii* implanted with melatonin underwent reproductive regression after 8 weeks of treatment. Individuals of this subspecies also displayed elevated lymphocyte proliferation to Con A compared to mice implanted with empty capsules. Conversely, *P. m. luteus* implanted with melatonin did not undergo reproductive regression and displayed no significant changes in lymphocyte proliferation. These data suggest that reproductive photoperiodic responsiveness, and more specifically, reproductive responsiveness to melatonin, mediates short-day enhancement of immune function in deer mice. These data also imply that melatonin may not possess universal immunoenhancing properties, and suggest that reproductive and immune responsiveness to day length are linked in these species. The effectiveness of melatonin to influence immune responses may be constrained by reproductive responsiveness to this indole-amine. The possibility also remains, however, that other immune parameters, not examined in these studies, are influenced by melatonin treatment.

As noted previously, melatonin has direct effects in vitro on immune function [33, 45]. The effects of melatonin in vivo are often difficult to assess because many hormones (e.g. prolactin, sex steroid hormones, gonadotropins, etc.) are altered by melatonin treatment (but see [43]). One way to approach this problem is to use an animal model in which reproductive function is not affected by melatonin. Melatonin or blank capsules were implanted in two groups of castrated starlings (*Sturnus vulgaris*) in one study [14]. One group was photorefractory and the other group was photostimulated; melatonin prevented the suppression of splenocyte proliferation in photostimulatory birds in the absence of changes in sex steroid hormones [14].

Few studies have reported the effects of photoperiod on immune function of short-day breeders (e.g. sheep, red deer). Humans may retain minimal reproductive responsiveness to day length [26]. The extent to which humans retain immunologic responsiveness to day length or melatonin remains unspecified. Thus, the importance of melatonin to human immune function or in the clinical treatment of cancer or other immunologic disorders remains unspecified (but see [28]). Melatonin appears to be part of an integrated system involved in coordinating reproductive, immunologic and thermoregulatory processes in nonhumans. Additional studies are required to understand the interactions among these physiological processes.

3. ENERGETICS OF IMMUNE FUNCTION

Mounting an immune response requires energy. The cascade of cellular events during the acute phase immune response and inflammation, and the elevation of body temperature in response to cytokine activation, presumably requires substantial energy, although precise quantification is lacking ([68, 99] but see below). Cytokine activation

elevates body temperature and the energy requirements of inflammation and acute phase immune responses may increase metabolic rates > 10 % per degree of body temperature elevation (reviewed in [98]). In one study of the energetic costs of mounting an immune response [42], house mice (*Mus musculus*) were injected with a specific antigen, keyhole limpet hemocyanin (KLH). This substance induces an antibody response without inducing fever or making the treated animal ill [44]. Both oxygen consumption ($mL \cdot kg^{-1}$) and metabolic heat production ($kcal \cdot kg^{-1}$) increased in KLH-injected animals. Thus, a general energy deficit can increase the risk of infection and death because insufficient energy reserves may be available to sustain immunity.

Stress compromises immune function (see [1, 47, 115] for reviews). Prolonged or severe food shortages may evoke secretion of glucocorticoid hormones [106]; glucocorticosteroids actively compromise aspects of immune function ([73, 99, 105] and see below), possibly by shunting energy from immunological processes to other systems needed for coping with stress [134].

3.1. Seasonal stressors and the effects of glucocorticoids on immune function

Many interactions between glucocorticoids and immune cell function have been reported in relation to environmental stress (reviewed in [105, 111]). However, the mechanisms underlying seasonal changes in stress hormones and immune function have not been elucidated. Adrenocortical hormones, especially glucocorticoids, suppress immune function in both humans and nonhuman animals [1, 12, 19, 35, 64]. Glucocorticoids are released in response to stressful stimuli, and can compromise cellular and humoral immune function [15, 77]. Adrenalectomy enhances lymphatic organ masses and B-cell activities [129]. The precise mechanisms by which the immune system is affected by the hypothalamic-pituitary-adrenocortical axis (HPA) are unknown, but probably involve the rate of cytokine release from activated immunological cells [16, 17]. Regardless of mechanism, substantial evidence links glucocorticoids with suppressed immune function.

Recently, a direct link between melatonin and glucocorticoid biology has been established. Generally, melatonin enhances immune function, whereas glucocorticosteroids compromise immune function [61, 91, 92 95, 99]. Melatonin treatment, however, can ameliorate the immunocompromising effects of glucocorticosteroids [2, 3, 22, 97, 117, 118]. Conversely, glucocorticosteroids can reduce the immunoenhancing properties of melatonin. For example, cortisol treatment of ducklings reduced the number of thymic melatonin receptors [123]. Proliferation of human lymhpocytes was reduced by approximately 50 % in the presence of cortisol; melatonin did not affect proliferation [131]. However, melatonin plus cortisol added to the media caused a further decline (~75 %) in proliferation rates [116]. The thymus gland possesses glucocorticoid receptors [117, 118].

Previous studies have demonstrated that environmental stressors elevate blood glucocorticoid levels and that high glucocorticoid levels suppress immune function [1, 12, 17, 19, 35, 64, 72]. For example, low ambient temperatures are often perceived as stressful, and can potentially compromise immune function (e.g. [35, 89, 104]). Winter survival in small animals is hypothesized to require a positive balance between short-day-enhanced immune status and glucocorticoid-induced immunosuppression [39]. This immunosuppression may be due to many factors, including overcrowding, increased competition for scarce resources, low temperatures, reduced food availability, increased predator pressure or lack of shelter. Each of these potential stressors may cause high blood concentrations of glucocorticoids (see below). Winter breeding with its concomitant elevation in sex steroid hormones may also cause immunocompromise

(e.g. [84, 141]). Presumably, winter breeding occurs when other environmental stressors such as temperature and food availability are not severe. The balance of enhanced immune function (i.e. to the point where autoimmune disease becomes a danger) against stress-induced immunosuppression (i.e. to the point where opportunistic pathogens and parasites overwhelm the host) must be met for animals to survive and become reproductively successful. Thus, the mediation of reproductive function and immune function will likely be intertwined [17].

Recently, the interaction between photoperiod and temperature was examined on antibody levels and splenic mass in male deer mice [39]. Animals were maintained in long or short photoperiods and either in 20 or 8 °C temperatures. Serum immunoglobulin G (IgG) concentrations were elevated in short-day mice maintained at normal room temperature as compared to long-day animals. Functionally, increased nonspecific IgG concentrations could reflect increased infection or could reflect increased immunosurveillance. Because sentinel mice housed in the same rooms were evaluated regularly and found to be pathogen-free, we surmised that increased IgG concentrations reflected increased immunosurveillance. Further work will have to be conducted to test this assumption. Long-day deer mice kept at temperatures of 8 °C had reduced IgG concentrations; mice exposed to short days and low temperatures had IgG concentrations comparable to long-day mice maintained at 20 °C. In other words, short days elevated IgG concentrations over long days. Low temperatures caused a significant reduction in IgG concentrations. The net effect of short-day enhancement and low temperature reduction of IgG concentrations is no appreciable difference from baseline (i.e. long-day mice kept at 20 °C). This adaptive system may help animals cope with seasonal stressors and ultimately increase reproductive fitness [41].

In addition to the well-established seasonal cycles of mating and birth, there are also seasonal cycles of illness and death among many populations of animals (e.g. [22, 23, 84, 102]). Because many stressful environmental conditions are somewhat recurrent, we hypothesize that animals have evolved mechanisms to combat seasonal stress-induced reductions in immune function. From an evolutionary and ecological perspective, it is reasonable to expect that animals have evolved the ability to forecast recurrent conditions associated with immunosuppression and bolster immune function in advance of these challenging conditions in order to maximize survival.

All laboratory studies of photoperiodic effects on immune function have reported enhanced immune function in short day lengths (reviewed in [111, 113]). Although many field studies support this hypothesis, with data suggesting enhanced immune function and decreased disease prevalence during the winter as compared to the summer, a substantial number of studies have reported the opposite pattern of results [111, 113]; i.e. immune function is compromised during the short days of winter. These conflicting results can be resolved by considering additional environmental factors, not usually manipulated in laboratory studies. For example, winter-associated stressors (e.g. restricted food and low ambient temperatures) appear to counteract short-day enhancement of immune function in the lab (reviewed in [39]). Thus, we predict that enhanced immune function should be observed during mild winters, whereas compromised immune function should be expected during challenging winters. Long-term field studies are required to test this hypothesis. Although the effects of melatonin on immunity are well-established (see [31, 56, 60, 119, 122] for recent reviews), an ecological context is needed to understand the effects of melatonin upon immune function, and to suggest why this phenomenon might be adaptive and functional, rather than merely a physiological oddity. Knowledge of the adaptive and functional significance of seasonal fluctuations in immune func-

tion may help to provide an improved understanding of the possibilities, as well as the constraints, of melatonin immunotherapy.

In order to examine the role of energetics in seasonal changes in immune function in deer mice, the chemical compound 2-deoxy-D-glucose (2-DG) was used to manipulate energy availability at the input end of the energetic equation [43]. 2-DG is a glucose analog that inhibits cellular utilization of glucose, thus inducing a state of glucoprivation [37, 136, 147]. 2-DG acts as a metabolic stressor, increasing serum corticosterone concentrations [88], and 2-DG glucoprivation induces an anestrous state in female Syrian hamsters (*Mesocricetus auratus*) [135] and torpor in female Siberian hamsters (*Phodopus sungorus*) [37]. 2-DG-induced metabolic stress also affects immune function; 2-DG administration inhibits murine splenic T lymphocyte proliferation in a dose-dependent fashion in laboratory strains of rats (*Rattus norvegicus*) [88] and mice (*M. musculus*) [103].

Short days buffered the animals against glucoprivation stress. Long-day mice injected with 2-DG had elevated corticosterone concentrations as compared to long-day mice injected with saline; corticosterone concentrations were not significantly elevated in short-day mice injected with 2-DG. This result could reflect an adjustment in the negative feedback of glucocorticoids on the HPA axis that is analogous to the enhancement of negative feedback of the HPG axis of rodents housed in short days [51]. In terms of immune function, 2-DG-treated long-day mice displayed reduced splenocyte proliferation to ConA as compared to control mice. Splenocyte proliferation did not differ among short-day deer mice regardless of experimental treatment; short-day animals exhibited enhanced immune function; short-day mice treated with 2-DG displayed higher splenocyte proliferation than long-day mice treated with 2-DG.

These data are also consistent with the hypothesis that short days buffer against metabolic stress. Reduced corticosterone levels in animals maintained on short days or treated with melatonin are likely due to improved metabolic function [133]. Accordingly, improved immune function in short days represents one component of the numerous winter-coping adaptations that are mediated by melatonin. The effect of altered energy states such as torpor on immune function is currently being studied in our lab. We are also interested in observing the effects of pregnancy and lactation on immune function when the dams are under different energetic constraints.

4. MELATONIN EFFECTS ON IMMUNE FUNCTION AND CANCER

4.1. Melatonin and immune function

There have been reports that melatonin both enhances or inhibits humoral and cellular immunity in mice and rats. Compounding the seemingly contradictory data are the observations that many laboratory strains of mice (e.g. C57BL/6, BALB/c and NZB) for which melatonin effects on immune function have been reported, lack functional melatonin receptors [48, 58]. Thus, the extent to which melatonin possesses universal immune-enhancing properties remains controversial (see [128]; cf. [96]). Laboratory strains of mice and rats are not reproductively responsive to photoperiod [27, 112]. Because historically mice and rats that failed to reproduce in a breeding colony (regardless of photoperiod) were culled, it is not unreasonable to expect that individuals that bred in unregulated photoperiods may have numerous atypical features in the components of the photoperiodic time measurement system that regulate breeding. However, it is also reasonable to suggest that nonreproductive traits that were unlikely to be selected against in a breeding colony may persist [112]. Thus, responsiveness of immune tissue or pelage may retain responsiveness to melatonin despite a lack of reproductive responsiveness to melatonin.

A number of studies have now confirmed the existence of melatonin receptors in lymphatic tissue, as well as on circulating cells of the immune system. For example, a series of studies has established the presence of 2-[^{125}]iodomelatonin binding sites in the spleen of birds and mammals (e.g. [4, 120–123, 126]). Binding sites on rat splenocytes were located in the cell nucleus, rather than membrane, and display reversibility, high affinity, specificity and light sensitivity, as well as time and temperature dependency [126].

Importantly, the affinity of the 2-[^{125}]iodomelatonin binding sites on splenocytes were consistent with binding at physiological concentrations of blood melatonin. Melatonin binding sites have also been found on human lymphoid cells [29]. Melatonin partially inhibited cyclic AMP production in human lymphocytes, but only at pharmacological doses [125]. As predicted [60, 93], membrane-bound melatonin receptors have been isolated on circulating lymphocytes and thymocytes [2, 3, 82, 85, 100, 115, 116, 120]. The melatonin receptors on lymphatic tissue appear similar in Kd values to melatonin receptors localized in rat and hamster brains, and also seem to be coupled to G-protein(s) [29].

Melatonin has been reported to counteract the immunosuppression after exposure to acute stress, drug treatment, certain viral diseases or during aging (reviewed in [60, 91, 92]). Melatonin also provides protection against gram-negative septic shock or hemorrhagic shock [28, 145, 146]. It appears that melatonin receptors have been located on T-helper-2 lymphocytes in the bone marrow. Activation of this putative melatonin receptor with both physiological or pharmacological concentrations of melatonin evoked production of interleukin-4 (IL-4) which induced hematopoietic growth factors secretion from bone marrow stromal cells [92].

Specifically, melatonin treatment of both normal and immunocompromised house mice elevates in vitro and in vivo antibody responses [31]. Impaired T-helper cell activities in immunocompromised mice are restored by melatonin treatment [31]. Antigen presentation by splenic macrophages to T-cells is also enhanced by melatonin; furthermore, this enhancement is coincident with an increase in MHC class II molecules, as well as IL-1 and TNF_{α} production [119].

Some of the older literature examining the effects of melatonin on immune function is inconsistent with the more recent literature. For example, in one early paper, neonatal pinealectomy was reported to be ineffective at evoking immune function change [71]. More recently, neonatal pinealectomy has been reported to affect immune parameters; murine antibody-dependent cellular cytotoxicity (ADCC) was reduced in adults that were pinealectomized before 7 days of age [143]. ADCC is a lytic process that occurs when lymphocytes bind to specific antibody-coated target cells through receptors for the Fc portion of the IgG molecule expressed on their membrane. The impairment in ADCC appears peripubertally, around 60 days of age, suggesting an involvement of sex steroid hormones [143]. Melatonin enhances ADCC [55]. Melatonin has been shown to enhance IL-2, IL-6 and inteferon-γ production by human circulating $CD4^+$ cells [53].

Melatonin may serve as an anti-inflammatory agent [81]. Pinealectomy ameliorates collagen II-induced arthritis in mice [63], and has been reported to inhibit humoral immune function and depress bone marrow progenitors for granulocytes and macrophages in mice [74]. Constant darkness enhances autoimmunity to type II collagen and development of collagen-induced arthritis in mice [62]. Low melatonin concentrations were observed 18 days after treatment with mycobacterioal Freund's adjuvant which induced inflammation of the joints in aged and young rats; melatonin replacement restored the inflammatory response in old rats to the level observed in young animals [30]. Melatonin also appears to inhibit NF-κ-B DNA-binding activity in

the spleen of rats during the night when endogenous melatonin secretion peaks; exogenous treatment during the light, when endogenous production of melatonin was low, caused a marked reduction in NF-κ-B DNA-binding activity [34]. In general, pinealectomy or constant light exposure inhibits T-cell autoimmunity by eliminating melatonin. Luzindole, a specific melatonin receptor antagonist, suppresses the development of experimental autoimmune encephalomyelitis induced by injection of spinal cord homogenate [36]. Additionally, NK cell activity and IL-2 production are reduced in mice after pinealectomy [38], and enhanced after melatonin treatment ([28] but see [78]).

In addition to its effects on autoimmune diseases, melatonin also has direct effects on other disease processes. For example, mink (*Mustela vison*) can suffer from a persistent parvoviral infection called Aleutian disease. Melatonin protects mink from Aleutian disease, as well as from distemper [50]. Melatonin also protects against septic challenge [146].

The circadian synthesis and release of melatonin likely modulate antibody response and alter tumorigenesis [55]. At the normal cellular level, melatonin is believed to affect antimitotic processes as well as cytotoxic activity [113]. In mice, the circadian synthesis and release of melatonin plays a significant immunomodulatory role. When the synthesis of endogenous melatonin is blocked, antibody production is depressed; in contrast, transplantation immunity is not affected by pinealectomy [94, 97]. Pharmacological and surgical pinealectomy also modulate other immune parameters including plaque-forming cells and blastogenic responses of spleen cells and thymus cells to various mitogens [13, 74]. Furthermore, elimination of melatonin synthesis by pinealectomy profoundly decreased the proliferation of bone marrow progenitors for granulocytes and macrophages (CFU-MG); the night-time peak of melatonin completely abolished CFU-MG proliferation [74].

Melatonin-treated hamsters displayed increased splenocyte proliferation responses to a polyclonal T-cell mitogen (Concanavalin A (ConA)), but displayed significantly reduced proliferation to the polyclonal B-cell mitogen, lipopolysaccharide (LPS) [33]. Thus, melatonin enhanced T-cell-mediated immune function while reducing antibody-mediated immune potential, suggesting that melatonin shifts the immunological balance from humoral to cellular immunity [33].

In summary, melatonin appears to enhance immune function in most cases. In common with reproductive responses mediated by melatonin, there may be a temporal component to the biological actions of melatonin.

4.2. Melatonin and tumorigenesis

Melatonin also appears to influence tumor growth. Both experimental and clinical reports suggest that there is a link between cancer development and pineal function [10, 20, 75]. However, in many of the tumor models used, it is not obvious to what extent immune function is involved in the development or maintenance of tumors. Experimental and clinical reports suggest that there is a link between cancer development and pineal function [10, 20, 75]. Pinealectomy of adult male rats results in an elevated mitotic index, as well as in an increase in the incorporation of ^{32}P into the DNA of the spleen, small intestines, liver and adenohypophyses. The pineal has been suggested to cause a deceleration of the cell division of different tissues [18].

The effects of melatonin on neoplastic cell proliferation depend on the type of neoplastic tissue examined. In general, melatonin appears to inhibit neoplastic cell proliferation in a dose-dependent way; that is, cloning efficiency diminishes as melatonin doses increase [11, 52, 69]. The data, however, appear to be contradictory [79]. The vast majority of studies suggest that mela-

tonin slows tumor progression or promotion. For example, pinealectomy accelerates the growth of transplanted melanoma in hamsters [61], of transplanted Walker 256 carcinoma in rats [6, 130], and of transplanted Yoshida sarcoma in rats [75, 76]. Furthermore, removal of the pineal enhanced the incidence of mammary adenocarcinoma in the rat induced by the chemical carcinogen 9,10-benzanthracene (DMBA), particularly when low doses were used [138]. However, when high doses of DMBA were used, there was no significant difference between pinealectomized or sham-operated animals in the incidence of mammary neoplasms [5]. Transplanted DMBA-induced mammary tumors grew more slowly after melatonin treatment as compared to control rats that did not receive melatonin [9].

Administration of melatonin to pinealectomized hamsters abolished the effect of pinealectomy on the growth of implanted melanoma [49]. Similarly, tumor development and tumor incidence decreased with the administration of exogenous melatonin to female rats treated with the chemical carcinogen DMBA [5, 138]. The inhibitory effect of melatonin on tumor growth was also demonstrated using a transplantable leukemia in mice, a transplantable mammary tumor in rats, and macrophage and lymphocyte metabolism in Walker 256 tumor-bearing rats (reviewed in [101, 113]). Melatonin also seems to boost the anti-tumor activities of IL-2 in humans with solid neoplasms [80].

Recently, melatonin has been reported to be effective in protecting against tumor initiation, through mechanisms that do not directly involve the immune system. The chemical carcinogen, safrole, evokes DNA-adduct formation in liver tissue. Both physiological [139] and pharmacological [140] doses of melatonin co-administered with safrole suppressed DNA-adduct formation in rat liver tissue. A dose-dependent effect of melatonin on DNA-adduct formation was reported, indicating that pharmacological doses (e.g. resulting in a serum melatonin level of 13 950 $pg \cdot mL^{-1}$) were extremely effective in preventing modification of DNA in response to safrole exposure [140].

In a study from our laboratory, adult female deer mice (*Peromyscus maniculatus*) were housed either in long (LD 16:8) or short (LD 8:16) days for 8 weeks, then injected with DMBA dissolved in dimethyl sulfoxide (DMSO) or with the DMSO vehicle alone [21, 110]. None of the animals treated with DMSO developed tumors in any of the experiments. Nearly 90 % of the long-day deer mice injected with DMBA developed squamous cell carcinoma within 8 weeks of injection. None of the short-day deer mice injected with DMBA developed tumors [110]. Small lesions developed at the site of injection; short-day females had less severe lesions and healed faster than long-day females. The role of estrogens in the photoperiodic responses were ruled out in a follow-up study. In another follow-up experiment [110], female deer mice were injected with a slurry of microspheres that contained either bromocriptine (CB154) or were empty. Suppression of prolactin with CB154 decreased tumor incidence from 55.6 to 24 % in long-day females 8 weeks after injection with DMBA. Silastic capsules that were filled either with melatonin or cholesterol were implanted into long-day female deer mice in another study [110]; 8 weeks later, females received either an injection of DMBA or DMSO, and then were monitored for 8 weeks. Approximately 66 % of females implanted with cholesterol and injected with DMSO developed histologically verified tumors. None of the melatonin-implanted mice developed tumors [110]. Taken together, these results indicated that photoperiod, mediated by melatonin, and possibly prolactin, can exert a functionally significant effect on immune processes and clinical disease. A seasonal influence of DMBA-induced mammary tumors was reported for rats under constant laboratory conditions; about 60 % of treated animals developed tumors during the spring/summer, but > 40 % of the females developed

tumors if treated during autumn [83]. These results are consistent with recent findings that short days increase natural killer cell activity in Siberian hamsters [148].

This brief review emphasizes that pineal melatonin affects immune function, influencing both humoral and cell-mediated immunity. Most of the available data suggest an anti-carcinogenic effect of melatonin, although there are a few studies suggesting a pro-carcinogenic effect of melatonin [69]. Again, the conflicting effects on immune function are reminiscent of the confusion surrounding the anti- and pro-gonadal effects of melatonin on reproduction. The confusion was resolved with the understanding that the timing of melatonin treatment provided the critical cue in organizing reproductive response. It remains possible that the anti-carcinogenic effects of melatonin depend upon a circadian rhythm of tissue responsiveness, but the timing of melatonin treatment is rarely considered (but see [29]).

5. CONCLUSIONS

Winter is energetically demanding and stressful; thermoregulatory demands usually increase when food availability decreases. Physiological and behavioral adaptations, including termination of breeding, have evolved among nontropical animals to cope with the energy shortages during winter. Presumably, selection for the mechanisms that permit physiological and behavioral anticipation of seasonal ambient changes have led to current seasonal breeding patterns for many populations. In addition to the well-studied seasonal cycles of mating and birth, there are also significant seasonal cycles of illness and death among field populations of mammals and birds. Energetically challenging winter conditions can directly induce death via hypothermia, starvation or shock; surviving these demanding conditions likely puts individuals under great physiological stress. The stress of coping with energetically demanding conditions may increase adrenocortical steroid concentrations that could indirectly cause illness and death by compromising immune function. Individuals would enjoy a survival advantage if seasonally recurring stressors could be anticipated and countered by bolstering immune function [137]. The primary environmental cue that permits physiological anticipation of season is daily photoperiod, a cue that is mediated by melatonin. However, other environmental factors may interact with photoperiod to affect immune function and disease processes. Immune function is compromised during the winter in field studies of birds and mammals. However, laboratory studies of seasonal changes in mammalian immunity consistently report that immune function is enhanced in short day lengths. To resolve this apparent discrepancy, we hypothesize that winter stressors present in field studies counteract short-day enhancement of immune function. Prolonged melatonin treatment mimics short days, and also enhances rodent immune function. Reproductive responsiveness to melatonin appears to affect immune function. In summary, melatonin may be part of an integrative system to coordinate reproductive, immunologic, and other physiological processes to cope successfully with energetic stressors during winter.

ACKNOWLEDGMENTS

Preparation of this review and funding of unpublished experimental data were supported by US Public Health Service grant MH 57535 and US National Science Foundation grant IBN 97-23420.

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