CHAPTER 3

Melatonin and Immunomodulation: Involvement of the Neuro-endocrine Network

C. Haldar[1,], S.S. Singh[1], S. Rai[1], K. Skwarlo-Sonta[2], J. Pawlak[2] and M. Singaravel[1]*

Abstract

Many neurotransmitters, neuroendocrine factors and hormone can drastically change immune function. Environmental stimulus to the nervous system affects the immune system and vice versa, essentially via the endocrine system. The present chapter deals with seasonal fluctuation in immunity, endocrine functions and the incidence of opportunistic diseases documented in a variety of species. Changes in immune functions appear to be mediated by day length (dark phase) and secretion of melatonin (MEL) from pineal gland. On the basis of findings, we may suggest that MEL secretion induced by short photoperiod acts as blaster to the immune function in winter to help the individuals to cope with seasonal stresses that would otherwise compromise immune function to critical levels.

The mechanisms underlying action of MEL involve a complex neuroendocrine network. Opioid peptides particularly (β-endorphin and Met-enkephalin, have been implicated as immunomodulators. MEL stimulates the production of Interleukin-2 and γ-interferon. It has been demonstrated that MEL could acts directly on the target cells through high-affinity G-protein coupled membrane-bound receptors (MT1 and MT2) described in primary and secondary lymphoid organs of various mammalian species. There is a reciprocal communication between the HPA (Hypothalamo-pituitary-adrenal) axis and the immune system. The precise mechanism by which immune system affects HPA axis is unknown, but it probably involves the release of diverse cytokines from activated immune

[1] Pineal Research Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005, India.

[2] Department of Animal Physiology, Faculty of Biology, Warsaw University, Miecznikowa 1, 02-096 Warsaw, Poland.

**Corresponding author*: Tel.: + 91 542 2307149 ext. 125(0), Fax: + 91 542 2368174. E-mail: chaldar@bhu.ac.in, chaldar 2001@yahoo.com

cells. MEL completely counteracts thymus involution and immunological depression induced by stress or glucocorticoid treatment.

Generally, thyroid hormone enhances immune function by promoting thymocyte maturation and differentiation. Receptors for thyrotropin (TSH) with many similarities to TSH-binding sites on thyroid cells have been found on lymphocytes. We have noted an interesting feature of the functional relationships between thyroxin and MEL supporting our idea of "Trade off" hypothesis between those two hormones in the control of the immune status. Testosterone generally suppresses the immune function. Castration resulted in increased immunity. Siberian hamsters and squirrels kept in short day, decreased estradiol level in females, which can be responsible for a winter decline in the immune activity suggesting again the major role of MEL as an immuno-enhancer.

INTRODUCTION

Maintenance of health depends to a significant extent on the ability of the exposed host to respond appropriately and, eventually, to adapt to environmental stressors. It is now well established that inappropriate or maladaptive response to such stressors weakens the body's resistance to other stimuli from the environment, such as pathogenic organisms, which have an impact on the body via redundant and reciprocal interactions between the body and the brain. Anatomically and functionally interconnected nervous, endocrine and immune systems utilize a large array of chemical messengers including hormones, cytokines and neurotransmitters, and express specific receptors able to recognize chemical messages sent by a particular component of this homeostasis keeping systems (Besedovsky and del Rey, 1996). It is, therefore, obvious that many neurotransmitters, neuroendocrine factors and hormones can drastically change the immune function, and on the contrary cytokines derived from immunocompetent cells can profoundly affect the central nervous system. As a consequence, any environmental stimulus to the nervous system will affect the immune system and vice versa, essentially via the endocrine system. In this conceptual basis it should not be surprising to note that the day / night photoperiod, which constitutes a basic environmental cue for any organism, can also influence the immune hematopoietic system. As for many other adaptive responses, a major mediator of this influence seems to be the pineal gland, which transduces: (1) the light / dark cycle into the circadian synthesis, and (2) release of melatonin (Yellon et al., 1999; Guerrero and Reiter, 2002).

The study of the immune system is quite common among clinicians and physicians, but the basic biologists usually examined the interactions between endocrine organs and immunity. Furthermore, complete understanding of the mechanisms underlying the immunological and hematopoietic action of many neurotransmitters and neurohormones, including melatonin is still a far cry. Even between the basic biologists, the interactions of the endocrine and immune systems, which protect the seasonal breeders from natural challenges, has received very little attention.

Seasonal Challenges to Immune System

Seasonal fluctuation in immune functions and the incidence of opportunistic diseases have been well documented in a variety of species including humans (Nelson and Drazen, 1999). The number of circulating leukocytes, spleen and thymus masses in mice (*Mus*), rats (*Rattus*), rabbits (*Lepus*), dogs (*Canis*), ground squirrels (*Citellus*), voles (*Microtus*), deer mice (*Peromyscus maniculatus*), cotton rats (*Sigmodon hispidus)* and humans were reported to be elevated during autumn and winter (Lochmiller and Ditchkoff, 1999). Environmental pressures might have led to the increased sophistication of vertebrate anticipatory (adaptive) immune system, perhaps due to the enhanced threat of infections in these complexes. In long living animals, the development of a finely tuned immune system with circulating effector cells was favored. The challenges of the environment also come from the variations in ecofactors (photoperiod, temperature, rainfall/humidity), which is responsible for providing food and shelter to improve the status of the animal for survival.

Tropical mammals and birds expressing seasonal diseases such as conjunctivitis and dermal infection during monsoon (high temperature and humidity) present a relationship between annual rhythm in melatonin and immune status (Haldar et al., 2001; Haldar and Singh, 2001). Moreover, in diurnal tropical rodent *Arvicanthis ansorgei*, living in environmental conditions where the annual day length does not change dramatically, the changes in the pineal arylalkylamine N-acetyl transferase (AA-NAT) activity and melatonin content were found recently (Garidou-Boof et al., 2005). The immune function in this species was not examined to date, however, it is well accepted that their seasonal reproductive cycle is controlled by the annual variations in day length and modulated by ambient temperature, rainfall and food availability. It can be supposed that due to seasonality in their resistance against pathogens, animals indicated the difference in their immune function efficiency.

Photoperiodic Modulation of Immune Function

Seasonal changes in the immune functions appear to be mediated by day length, since in deer mice and Syrian hamsters, short-day exposure increases splenic mass and elevates total lymphocyte and macrophage count (Nelson and Drazen, 1999), as well as attenuates the development of infections (Bilbo et al., 2002). Also, in laboratory strains of rats (*Rattus norvegicus*), which are traditionally considered to be reproductively non responsive to photoperiodic information (Nelson and Blom, 1994), seasonal changes in immunity could be observed. Maintaining rats in constant darkness (DD) for four weeks increased thymic mass and the number of thymocytes, in comparison to the control animals maintained in normal day length (Mahmoud et al., 1994). Contrarily, constant light (LL) exposure for four weeks decreased the thymic mass in Wistar rats (Mahmoud et al., 1994). Wurtman and Weisel (1969)

reported that photoperiod also influences the splenic weight in rats. However, photoperiod appears to be more effective in influencing the immune function in seasonal breeders like hamsters or deer mice, i.e., species in which reproduction strongly depends on changing lighting conditions (Demas et al., 1996; Nelson et al., 1998). For example, splenic mass, total splenic lymphocyte number and macrophage count as well as white blood cell (WBC) number, were significantly higher in hamsters exposed to short-day, as compared to animals exposed to long photoperiod (Brainard et al., 1987). However, in this species, photoperiod neither affected thymic weight nor antibody production (Brainard et al., 1987). Deer mice maintained in short photoperiod displayed faster healing rates than long-day animals (Nelson and Blom, 1994). The pineal gland and its principal hormone, melatonin can also affect lymphatic tissue sizes. Exposure of male and female hamsters to short-days or daily afternoon melatonin injections elevated splenic mass, which could be prevented in short-day hamsters by pinealectomy (Guerrero and Reiter, 2002).

The Pineal Gland-Immune Network

The study of the two-way relationship between the pineal gland, melatonin and immune system has raised considerable importance in recent years (Guerrero and Reiter, 2002; Skwarlo-Sonta, 2002; Skwarlo-Sonta et al., 2003; Carillo-Vico et al., 2005). Experiments have shown that there is a functional relationship between the pineal gland and the immune system. Circadian rhythms are present in most, if not all, immune functions e.g., the peak level (acrophase) of circulating lymphocytes is observed close to that of melatonin in normal (24 hr) environment (Haldar et al., 2006). It has been reported that the circadian rhythm of mononuclear cells, T and B lymphocytes and the serum levels of melatonin, particularly affect the functionality of the natural killer cells in rats (McNaulty et al., 1990). Both surgical pinealectomy (Csaba and Barath, 1975; Del Gobbo et al., 1989), and functional (permanent lighting) and pharmacological (evening administration of the β-adrenergic blocker, propranolol) inhibition of the pineal gland function in mice (Maestroni et al., 1987) resulted in a depressed cellular and humoral immune response and IL-2 production. Exogenous melatonin administration in the evening reversed this effect, suggesting that circadian melatonin synthesis is mandatory for optimal immune functions (Rai and Haldar, 2003).

Moreover, a parallel pattern of the diurnal rhythms of melatonin and thymic hormones (thymosin α_1 and thymulin) was demonstrated in rats and humans. Pinealectomy caused a decrease in both hormone content in rat thymus and serum, reversed by melatonin injections (Molinero et al., 2000). The pineal gland exerted a stimulatory action on thymic growth, since the administration of pineal extracts might cause in the peripubertal mice, around 60 days of age, a thymic hyperplasia, suggesting an involvement of sex steroid hormones (Vermeulen et al., 1993). Disruption of the nighttime peak

of melatonin following pinealectomy completely abolished the proliferation of bone marrow progenitors for granulocytes and macrophages (colony-forming units granulocyte-macrophage (CFU-GM)); Haldar et al., 1991, 1992a, b, c). As predicted, melatonin receptors have been identified on circulating lymphocytes (Calvo et al., 1995), thymocytes and splenocytes of rats (Rafii-El-Idrissi et al., 1995) and Indian palm squirrel (Rai, 2004), suggesting a direct effect of melatonin on the immune system function.

On the basis of findings, it could be suggested that melatonin secretion induced by short photoperiod, acts as blaster to the immune function of these animals in winter to help the individuals to cope with seasonal stresses (low ambient temperature) that would otherwise compromise the immune function to critical levels. On the other hand, in seasonally breeding and hibernating Siberian hamsters *Phodopus sungorus*, field studies showed decreased immune activity during short-day period of the year (autumn and winter), in contradiction to laboratory studies, in which many immune parameters are elevated in short-day (Nelson et al., 1995). In the authors' model of experimental peritonitis, hamsters kept in short-day laboratory conditions presented a decreased inflammatory reaction measured as the reactive oxygen species (ROS) production in peritoneal leukocytes compared to long-day animals. Also, splenocyte proliferation in the latter was significantly higher (Pawlak et al., 2005). Melatonin addition *in vitro* further inhibited both ROS production and splenocyte mitogenic response. These data suggest that the influence of melatonin on the immune system depends on the species and experimental model used. It shows also that mechanisms underlying its action in the immune system involve a complex neuroendocrine network.

In one of the authors' experimental models, the tropical Indian palm squirrel *F. pennanti*, short-day related increase in immunity was noted, suggesting a strong influence of endogenous melatonin in maintenance of immune status in this seasonal breeder (Haldar et al., 2001; Rai, 2004). The authors further interested in immune status of seasonally breeding laboratory animals, when observed at a certain part of the year they are susceptible to more infections and diseases and these are particularly the transitional phases between two reproductive periods i.e., progressive and regressive phases (Singh, 2003; Rai, 2004). Indian palm squirrels, during the reproductively active phase in summer months, are healthy even though the peripheral level of the so-called immunoenhancing hormone-melatonin, is low. The favorable environmental conditions with enough food, shelter for young ones and photoperiod along with internal high gonadal steroids could also be responsible for their good health. On the other hand, during reproductively inactive phase when the environmental conditions are not that favorable for squirrels, the internal high melatonin enhances the immune function to keep them healthy and to surpass winter (Haldar et al., 2004). Transitional periods, however, are crucial for this small rodent i.e., gonadal regressive and progressive phases when neither melatonin is having a threshold to enhance

immunity, nor are the decreasing gonadal steroids. Work is in progress to examine the sensitivity of melatonin receptors on the lymphoid tissues during those two transitional phases and to find means to improve the immune status of the squirrels. The findings clearly revealed that endogenous melatonin had a positive effect on rhythmic function of some immune parameters such as CFU-GM of bone marrow (Haldar et al., 1992a, b, c). The authors accessed the effect of melatonin on immune parameters in a seasonally breeding animal, the Indian palm squirrel *F. pennanti* and bird *P. asiatica* during reproductive phases *in vivo* as well as *in vitro*. Melatonin treated squirrels and birds showed an increase in percent lymphocyte count (% LC) and peripheral blood, total leukocyte count (TLC) and percent stimulation ratio (%SR) of thymocytes and splenocytes, which was low following pinealectomy in squirrels. Melatonin administration recovered all the decreased immune parameters to the control level (Rai and Haldar, 2003). MEL supplementation also enhanced all immune parameters in both *in vivo* and *in vitro*, suggesting that the immune system was sensitive during the reproductively active phase to MEL when peripheral melatonin level was low and the role of melatonin could be important for the maintenance of the immune status of seasonal breeders.

MECHANISM OF IMMUNOMODULATION BY MELATONIN

The mechanism by which melatonin modulates the immune function is still not resolved. However, the following hypotheses have been suggested:

1. The Pineal Gland–Immune System – Opioid Network

Opioids have been suggested to be the mediators of melatonin action on the immune system. Naltrexone – the opioid antagonist, abolished the immunoenhancing and antistress effects of melatonin (Maestroni et al., 1999). Dynorphin 1-b and β-endorphin mimicked the immunological effects of melatonin. Melatonin also stimulated the release of opioid peptides by activated T-helper thymocytes (Maestroni and Conti, 1990). These melatonin-induced immuno-opioids (MIIO) mediated the immunoenhancing and antistress effects of melatonin and cross-reacted immunologically with anti β-endorphin and anti-met-enkephalin antisera in laboratory rodents (Maestroni and Conti, 1990). Recently, proopiomelanocortin (POMC) and enkephalin genes expression in immune cells (peritoneal leukocytes and splenocytes) in exogenous melatonin treated chicken with experimental peritonitis have been demonstrated (Majewski et al., 2005).

Opioid peptides particularly β-endorphin and Met-enkephalin, have also been implicated as immunomodulators, since they can affect several immune mechanisms. However, it is difficult to conclude whether, as a whole these peptides are immunosuppressive or immunostimulant since the effects reported differ depending on the type of immune process studied, the cell source and type, the species, the concentrations of the opioid peptide used,

and the experimental conditions i.e., *in vivo* or *in vitro* (Homo Delarche and Durant, 1994). Actually, melatonin-induced endogenous opioid demonstrated in chickens seem to be responsible for a pro-inflammatory effect observed in this experimental model (Majewski et al., 2005).

2. Lymphokines as Mediators of Melatonin Action on the Immune System

The production of Interleukin-2 and γ-interferon is stimulated by melatonin (Caroleo et al., 1992). As interleukins and interferons are known stimulators of natural killer cells' activity and / other immune cells, it is possible that these lymphokines mediated the observed effects of melatonin on the mammalian immune responses. Recent data presented a synergistic effect of melatonin and lipopolysaccharide conditioned medium (containing induced IL-1), on the T-cell genesis (thymus) and also on functional B and T lymphocytes (spleen). An explanation could be that initially melatonin binds to specific melatonin receptors in helper T cells and / or monocytes stimulating the production of either IFN- γ, IL-2, melatonin induced immuno opioid (MIIO), IL-1, IL-6 and IL-12, which in turn might upregulate the immune response. However, these explanations to be studied further to note their balancing effect on immune status under adverse environmental conditions (Haldar et al., 2004).

3. Direct Melatonin Action

a. Melatonin Receptors within Immune System

Different aspects of melatonin effects within immune system was extensively reviewed and discussed by Carillo-Vico and co-workers (2005). It has been demonstrated that melatonin acts directly on the target cells through high-affinity G-protein coupled membrane-bound receptors (Dubocovich and Markowska, 2005). This type of receptor has been described in:

Primary and secondary lymphoid organs of various mammalian species (Lopez–Gonzalez et al., 1993; Poon et al., 1994).

Rat splenocytes (Rafii-El-Idrissi et al., 1995).

Human peripheral lymphocytes.

T-helper lymphocytes in bone marrow (Maestroni, 1995).

Splenocytes, thymocytes and bone marrow lymphocytes of Indian palm squirrel (Rai, 2004).

Moreover, being a highly lipophilic molecule, melatonin easily penetrates all biological barriers, including cell membrane, and therefore is able to exert its action also within immune cells, using RZR / ROR (retinoid Z receptor / retinoid orphan receptor) orphan nuclear receptors, described also in immune cells (Rafii-El-Idrissi et al., 1998). These receptors seem to mediate some effects of melatonin on cytokine production, cell proliferation and oncostasis (Garcia-Maurino et al., 2000; Winczyk et al., 2001; Treeck et al., 2006).

b. Melatonin as an Antioxidant

Melatonin is also well-known as a potent antioxidant due to its particular property of free radical scavenging (Reiter et al., 2000a), demonstrated also within the immune system (Reiter et al., 2000b). Thus, melatonin exhibits a strong anti-inflammatory activity, counteracting liposachharide (LPS)-induced nitric oxide synthase (NO production) and abolishes a rise in lipid peroxidation in both *in vivo* and *in vitro* inflammation models. It has been also demonstrated that melatonin protects against oxidative damages through the stimulation of anti-oxidative enzymes, i.e., reduced glutathione level (Gitto et al., 2001). Melatonin also inhibits anti-inflammatory cytokines (e.g., IL-12) and pro-inflammatory cytokines (like TNF-α, IL-8) as well (Reiter et al., 2000a).

4. Melatonin Action through Neuro-endocrine Network

It is now considered that both neural and endocrine factors work together to maintain the immune system within a safe operating limit, to prevent the over activation of the immune system to avoid the destruction of self tissue and cells (Fig. 1).

(a) Hormones of the Hypothalamo-pituitary-adrenal Axis

When the hormonal regulation of the immune system is considered, the main focus should be on glucocorticoids, which have a well-documented

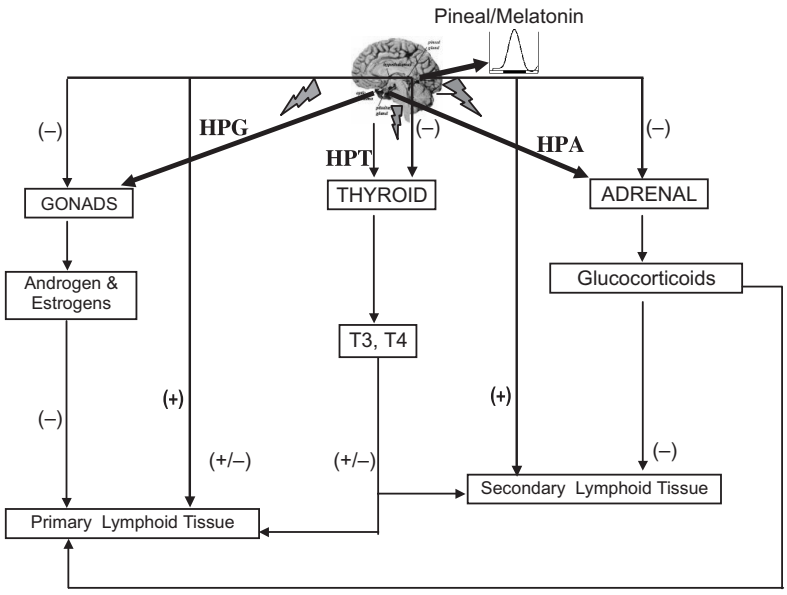

Fig. 1. Pineal-endocrine control of immune system (HPG – hypothalamo-pituitary-gonadal, HPT – hypothalamo-pituitary thyroid and HPA – hypothalamo-pituitary-adrenal axis).

immunoinhibitory activity (Black, 1994). Adrenalectomy increases lymphatic organ masses and B-cell activity (del Rey et al., 1984). Corticoids can act directly on immune cells through their receptors (Werb et al., 1978) reducing, for example, production of cytokines (Munk and Guyre, 1986). However, the upstream components of the hypothalamo-pituitary adrenal (HPA) axis, such as adrenocorticotropic hormone (ACTH) and corticotropin releasing hormone (CRH) may affect immune functions, i.e., ACTH is known to influence immune cell functions *in vitro* (Audhya et al., 1991).

There is a reciprocal communication between the HPA axis and the immune system. Increased glucocorticoid production is associated with the activation of the immune system by numerous antigens (Besedovsky et al., 1991). Moreover, activation of the immune system not only stimulates the activity of HPA axis as part of a regulatory response, but there is evidence that immune cells themselves may produce endocrine products such as, ACTH (Smith and Blalock, 1981), which acts locally as an immunoregulator. The precise mechanism by which the immune system affects HPA axis is unknown, but it probably involves the release of diverse cytokines from activated immune cells (Besedovsky and del Rey, 1996).

Glucocorticoids are released under stressful situations, such as unfavorable environmental conditions, and can compromise cellular and humoral immunity (Levi et al., 1988). Also, circadian variations in several immune parameters are negatively correlated with adrenal hormone secretion (Angeli et al., 1992). The HPA axis may be involved in the mediation of melatonin action on the immune system. Melatonin treatment can ameliorate the immunocompromising effect of glucocorticoids (Aoyama et al., 1987; Haldar et. al., 2004), however, the immunostimulatory action of melatonin also varies with the steroid milieu (Persengiev et al., 1991), suggesting a bi-directional interaction between the two. For example, cortisol treatment of ducklings reduced the number of thymic melatonin receptors (Poon et al., 1994) and melatonin treatment decreased the density of thymic glucocorticoid and progestin receptors in rats (Persengiev et al., 1991).

With the demonstration of a steroid-dependent modulation of the rat thymic glucocorticoid receptors by melatonin, and modulation of the duck thymic 2[^{125}I] iodomelatonin binding sites, the thymus has been suggested to be the target site for the immunomodulatory interactions between pineal melatonin and adrenal steroids (Persengiev et al., 1991; Poon et al., 1994). Melatonin may completely counteract thymus involution and immunological depression induced by stress or glucocorticoid treatment (Maestroni and Conti, 1990).

The results of the authors' experiments on Indian ground squirrels showed that long-term (60 days) treatment with dexamethasone, which can be compared to long-term natural stress, causes a significant decrease of immune status and plasma melatonin level in these animals (Haldar et al., 2004). However, significant restoration of immunity (i.e., antibody production) and plasma melatonin level was found in simultaneous dexamethasone and

melatonin treated animals. On the other hand, it was observed that in Siberian hamster, short-day (prolonged melatonin synthesis) caused a significant increase in plasma cortisol level, which, in turn, might have affected the activity of the immune system of these animals (Pawlak et al., 2005). Taken together, these results suggest that melatonin may act on two different levels – as a signaling molecule transducing the information about the shortening day length, in which case it would inhibit the immune activity by stimulating the release of stress hormones, or directly on immune cells as an immunoenhancer, probably through the increased synthesis of MIIO by immune cells. This explanation is further supported by the fact that the administration of exogenous melatonin had a similar effect as exposure of hamsters to short-day and resulted in the increased aggression in these animals (Jasnow et al., 2002).

(b) Hormones of the Hypothalamo-pituitary-thyroid (HPT) Axis

Thyroid hormones, like glucocorticoids, are important for animal adaptation to changing environmental conditions, especially temperature. Thyroid activity of non-hibernating mammals increases during winter compared to summer, in contrast to hibernating mammals, where it decreases during winter (Tomasi et al., 1998). Generally, thyroid hormones enhance immune function, for example, by promoting thymocyte maturation and differentiation (Gala 1991; Fabris et al., 1995). This effect is probably modulated by the thymic peptide – thymulin, as thyroidectomy reduces the thymulin concentration and thyroid hormone replacement restores it to control values (Fabris and Mocchegiani, 1985). Several human disorders characterized by reduction in thyroid hormones are also associated with reduced thymulin concentrations accompanied by immune deficiency (Fabris et al., 1995). In healthy human populations, however, T_3 appears to have a direct stimulatory effect on B-cell differentiation (Paavonen, 1982). Also, upstream components of the HPT axis influence immune function. Receptors for thyrotropin (TSH), possessing many similarities to TSH-binding sites on thyroid cells, have been found on lymphocytes (Pekonen and Weintraub, 1978). In addition, TSH enhances the immune response to specific antigens: it stimulated, for example, the antibody response to T-cell dependent and T-cell independent antigens *in vitro* (Kruger and Blalock, 1986). However, TSH alone does not enhance proliferation independent on antigen stimulation (Provinciali et al., 1992). HPT axis also seems to be responsive to seasonal fluctuations in melatonin levels and might be involved in mediating seasonal changes in immune functions. Melatonin is known, for example, to stimulate the release of hypothalamic thyrotropin-releasing hormone (TRH), which possesses immuno-reconstituting and antiviral activity (Pierpaoli and Yi, 1990). It may also be speculated that the effects of thyroid hormones are secondary to those evoked by steroid hormones or melatonin. It has been demonstrated that the thyroid gland and HPA axis can modulate each other's activity, restoring to some extent their proper functions during different

abnormalities. Thus, thyroid hormones may affect immune function by reducing HPA axis activity, which in turn suppresses the immune function.

The thyroid is probably involved in the regulation of immune function/status in Indian palm squirrels, as thyroidectomy in *F. pennanti* decreased weight of thymus and spleen, TLC, percentage LC of blood and bone marrow, and percentage stimulation ratio of thymocytes and splenocytes, and these parameters were restored to control levels after treatment with melatonin. Moreover, an interesting feature of the functional relationship between thyroxin and melatonin has been noted during two different reproductive phases (i.e., active and inactive), which supports the authors' idea of "Trade off" hypothesis between these two hormones in the control of the immune status. During the reproductively active phase, peripheral melatonin level was low while thyroxin was high, hence thyroxin might have acted as mitogen for lymphoid tissues. On the other hand, during the reproductively inactive phase, when thyroxin level was low and melatonin was high, probably melatonin acted as an immuno-enhancer. Therefore, a complete "Trade off" interrelationship between thyroxin and melatonin was proposed as a mechanism maintaining high immune activity during both phases for the benefit of survival of animals (Fig. 2; Rai et al., 2006).

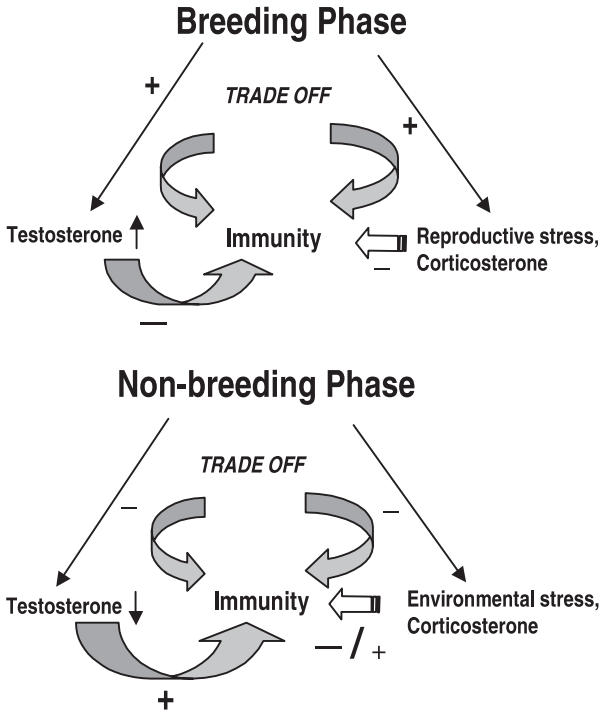

Fig. 2. Hypothetical diagram showing trade-off interrelationship between steroid and melatonin during breeding and non-breeding phase.

(c) Gonadal Steroids and Immunomodulation

The effect of gonadal steroids on lymphoid tissues is well known (Hammar, 1929). Even before the importance of the thymus in immune function had been recognized, researchers noted thymic hypertrophy in response to gonadectomy, particularly in female animals (Eidinger and Garrett, 1972). Consequently, receptors for estrogens and androgens have been found on lymphoid tissue, suggesting that the gonadal steroids can act directly on immune organs (Barr et al., 1984). Most species exhibit a seasonal pattern in reproductive behavior and physiology, including humans (Bronson, 1995), which can be correlated with seasonal variations in immune status (Bilbo and Nelson, 2003). In anticipation of winter or in laboratory-stimulated winter conditions, most rodent species exhibit a dramatic decline in gonadotropin and gonadal steroid concentration, eventually leading to the regression of the reproductive system (Bronson and Heideman, 1994; Bronson, 1995).

As with the HPA axis, numerous interactions exist between the HPG axis and the immune system. Importantly, sex steroid hormones are likely mediators of seasonal patterns of immune function (Fig.1). Testosterone generally suppresses the immune function (Singh and Haldar, 2005). Castration of adult male rodents results in increased humoral and cell mediated immunity, as well as increased lymphatic organ size, including thymic, splenic and lymph nodal masses (Schuurs and Verheul, 1990). Treatment of adult castrated males with physiological doses of testosterone compromised their immune function to pre-castrated levels. During short photoperiod, blood androgen level decreases; hence short photoperiodic treatment is similar to a functional castration and simultaneously increases peripheral melatonin level (Schuurs and Verheul, 1990). Thus, enhancement of immune function could be due to both, the removal of the immunosuppressive effects of the androgens and immunoenhancement by melatonin.

The effects of physiological doses of estrogens appear to enhance immune function. Blood estrogen levels are low in short-day females with high circulatory levels of melatonin (Haldar and Singh, 1995). Also, in Siberian hamsters kept in short-day, a significant decrease in estradiol level in females was observed, which can be responsible for a winter decline in the immune activity noted in these animals (Pawlak et al., 2005). On the other hand, the enhancement of winter immune function observed in squirrels is unlikely to involve photoperiod-mediated changes in estrogen levels, again suggesting the major role of melatonin as an immunoenhancer in this species.

CONCLUSION

Immune responses had been studied only in laboratory animal model where the involvement of season and other endocrine organs were scarcely discussed. The study of immune status of wild seasonally breeding species is highly important to save some endangered fauna leading to a new area of

research "Immunoecology". The hormones of adrenal (glucocorticoid) and thyroid (L-thyroxine) have some direct modulatory effects on immune status. Not only the gonadal steroids influence immune function, but the products of immune system also modulate the HPG axis. This reciprocal cross talk allows the maintenance of both the immune system and endocrine systems to be regulated within narrow limits. This provides a condition of "Trade off" interrelationship between immune factors and the HPG axis. This hypothesis may reply to some of the clinical problems of patients with chronic immune disorders experiencing gonadal abnormalities or dysfunction. In certain cases of immune disorders, melatonin can act as an immunoenhancer, hence may be of high clinical value.

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