Proc. zool. Soc., Calcutta, **59**(1) 87–104 (2006)

Immunomodulatory role of melatonin: Relationship with bone marrow macrophages and lymphocyte

Chandana Haldar* and Seema Rai

Pineal Research Laboratory, Department of Zoology
Banaras Hindu University, Varanasi-221 005, India

The immune system is not only driven by cytokines but also by hormone and neurohormones. Among neurohormones popularity of melatonin in immunomodulation is a current topic of research. This is because of rhythmic secretory pattern of melatonin and its inverse correlation with breeding activity and direct relation with immune status. In the present review we wish to update the current knowledge of melatonin as immunomodulator specially in seasonally breeding mammals because of an adaptive significance of melatonin in balancing immune status. It acts during winter as immunostimulator to save the seasonally breeding mammals from the adverse condition of the environment (low temperature, scarcity of food and shelter, etc) and free radical load. We proposed the mechanism of immunomodulation by melatonin and believe that it is the direct one in modulating the immunity according to the season. However, interdependence of melatonin with other endocrine gland (adrenal and thyroid) is well known and such a correlation of melatonin with endocrine system in regulation of immune function may throw some light to the clinical problems of endocrine dysfunction and immune deficiency.

Key words: Melatonin, bone marrow macrophages, lymphocytes, free radicals, melatonin receptor

Introduction

Melatonin (N-acetyl-5-methoxy-trptamines), the main indoleamine secreted by the pineal, exhibits a circadian secretory rhythm that conveys environmental information, particularly about photoperiod to the organism. Peak melatonin secretion occurs during the night, making melatonin the "*chemical messenger of darkness*" (Reiter, 1991). Melatonin is a small and lipophilic molecule rapidly diffusing throughout the body and modulating various physiological processes. During the last two decades the pineal gland became an organ of potential interest, when it was noticed that it plays an important role in the annual cycles of reproduction in vertebrates. Today, melatonin effects are much wider than previously suspected and indeed involve every cell in the organism. It has been shown to influence nearly every biochemical, physiological,

Email- chaldar2001@yahoo.com, chaldar@bhu.ac.in

endocrinological and behavioral pattern (Armstrong and Redman, 1991).

Within a decade of its discovery, melatonin had been shown to be functionally related to neuroendocrine physiology, with the most obvious link being its control of reproductive physiology in photoperiodic rodents (Hoffman and Reiter, 1965). A wonderful scheme defining the role of pineal gland, seasonally changing photoperiods and annual fluctuations in reproductive competence had been presented for seasonally breeding mammals (Arendt, 1988). The role played by melatonin is analogous to that played by light in the external *milieu* (Nir, 1995). In the last two decades, it has been suggested that the pineal gland may act as a neuroendocrine transducer, able to convey environmental information into hormonal signals, mainly through the synthesis and release of its hormone melatonin, which is involved in the modulations of several neuroendocrine activities (Reiter, 1982).

The immune system was believed, to be a self–contained endogenously regulated unit modulated by cytokines. It is now seem to be closely integrated with other physiological circuits, such as the central nervous systems and the pineal gland being one of the integral constituent (Gupta, 1990; Fig. 1). The pineal utilizes the photoperiod to alter its synthesis of melatonin and has been implicated as a major participant in immunomodulation (Poon *et al*., 1994; Giordano *et al*., 1993; Pioli *et al*., 1993). In order to characterize the immunomodulatory role of melatonin, researchers have studied the inter-relationship in this regard on the laboratory animal such as rat, mice and hamster (Maestroni *et al*., 1994 b; Nelson and Demas, 1996; Nelson *et al*., 2002).

Immunity and Seasonality

Immunity, a primeval feature of living organisms is essential for reproduction and to face the challenges originating from seasonal variation and environmental threats. Immunological resistance to the environment threat require energy and thus a general energy deficit can increase the risk of infections and death because of insufficient energy reserves available to

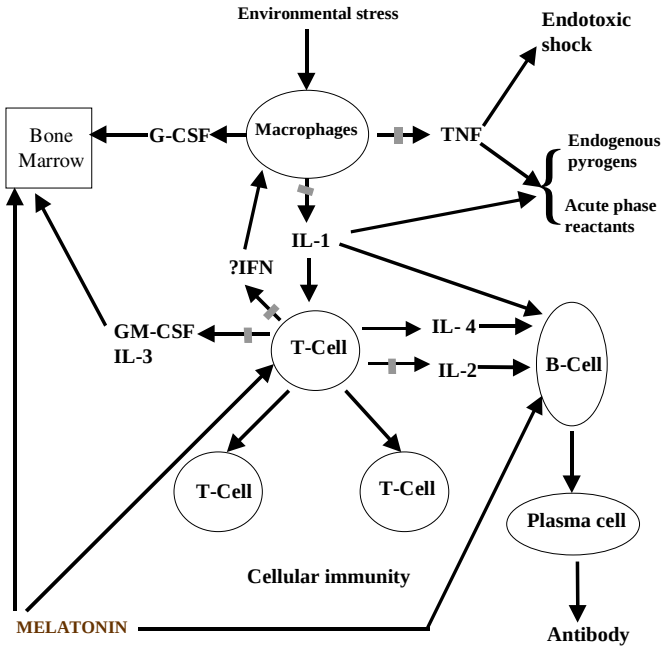

Fig. 1. Regulation of cellular and humoral response by glucocorticoids and action of melatonin

sustain immunity. Several stressful environmental condition such as reduced food availability, low ambient temperature, overcrowding, lack of shelter, or increased predator pressure, can recur seasonally, leading to seasonal fluctuation in immune function among individuals and seasonal changes in population – wise - disease and death rates. Those species, which modify their physiological state on an annual basis, are believed to use the pineal gland and its hormone melatonin to prepare and respond to upcoming seasons (Fig. 1).

We have proposed that the immune status of tropical animals show a seasonal rhythmicity (Haldar and Singh, 2001), which is essential for adaptation. The squirrel is a semi-wild seasonal breeder of tropical zones, which faces maximum challenges from the nature in winter (lack of food, shelter and with several seasonal diseases such as dermal and eye infections).

Our study reveals that the basal levels of immune parameters (total leukocyte count (TLC), Lymphocyte count (LC), % LC peripheral blood and bone marrow,) during reproductively inactive phase were higher than the reproductively active phase. The peak value of immune parameters in day time were favored by low plasma steroids and slightly high day time basal level of plasma melatonin in circulation whereas, the peak value of plasma level of melatonin in night time favored the increase in the immune parameters during reproductively inactive phase. This could be due to a stronger winter stress adaptation as reported by Kliger *et al.* (2000) for other rodents. Further, the immune parameters were enhanced in short days to counteract stress-mediated immune suppression occurring during winter in squirrels as already reported by Demas and Nelson (1996) for mice and hamster.

During the reproductively active phase, very less immune activities were observed but it showed a parallel rhythmicity with the low plasma melatonin. During reproductively active phase plasma level of testosterone was very high hence, suppressing the immune parameters during the daytime, while at nighttime a small-elevated peak of immune parameters occurred might be due to the increase in the plasma melatonin level. However, favorable environmental condition of summer (amount availability, shelter, low frequency in diseases) and high gonadal steroid in plasma protected the squirrels from seasonal infection and reduced the rate of mortality.

It is suggested by Bentley *et al.* (1998) that there is direct effect of reproduction on immune status. Hence in this rodent reproduction and immune status might have co-evolved as the steroid hormone influences reproduction and immune function both. Our data suggest a multiple and highly complex effects of endogenous melatonin in regulation of the rhythmicity of immune parameters (TLC, LC, % LC of peripheral blood and bone marrow) to contribute to homeostasis of immune system. It can be anticipated that melatonin acts on immune cells at several levels and in several ways direct or indirect by secreting a novel putative opioid like

peptide, melatonin-induced-immuno-opioid (MIIO) as suggested by Maestroni and Conti (1990). During reproductively inactive phase short photoperiod induced melatonin secretion acting as a blaster for the immune status in the winter phase to help the squirrel to combat with seasonal stressor (low temperature, lack of food, shelter) that would otherwise compromise immune function to critical level while, during reproductively active phase steroid hormone like thyroxin and also other eco-factors (high temperature, long photoperiod and amount availability of food) helped the squirrels to remain healthy. Therefore, it may be said that pineal gland and its hormone melatonin acts like a major temporal synchronizer to maintain a humoral and immune adaptability of this tropical rodent in a rhythmic manner.

The Indian palm squirrel, *F. pennanti* is a seasonal breeder. Its breeding season extends from April to July. In seasonally breeding rodents the reproductive function provides adjustment that permits individual to maintain a positive immune status despite a low levels of melatonin and high level of gonadal steroids. This adaptation is provided by positive ecological factors (temperature, photoperiod and humidity) and high food availability during reproductively active phase of this squirrel. There is a good correlation of seasonal fluctuation in immune function along with seasonal changes with disease and death rates. Reproductive fitness and positive environmental conditions lead the squirrels remaining healthy in reproductively active phase even with a "low" immune status.

Melatonin and daily rhythm in immunity

In contrast to melatonin, which peaks during the night both in diurnal and nocturnal species, the cycles of other hormones and several immune parameters correlates with the pattern of the animal locomotor activity resting (Demas and Nelson, 1996).

The peak value of plasma melatonin during the dark period always coincided with the increased immune parameters during reproductively active and reproductively inactive phases of the squirrel *F. pennanti* a tropical seasonally breeding rodent. In this animal model we have noted the peak plasma melatonin level during the dark photoperiod at ~2.00 hrs and in daytime a small melatonin peak was noted at 14.00 hrs of reproductively inactive phase (Sharma, 2002). These peaks are also observed in reproductively active phase having smaller amplitude than plasma melatonin of reproductively inactive phase (Bubenik, 2002). The melatonin level of *F. pennanti* (Haldar, 1996) being slightly high at 14.00 hrs could be due to afternoon *siesta* and energy balance (Bubenik, 2002) which decreased again at 18.00 hrs during winter and non significantly in summer as the basal melatonin level was already low.

Bone Marrow Macrophages

The immune status of *F. pennanti* an Indian tropical rodent can be judged by observing the cell density of spleen and thymus in histological sections along with circulating level of lymphocyte in peripheral blood and bone marrow. However, bone marrow, an important tissue in generation

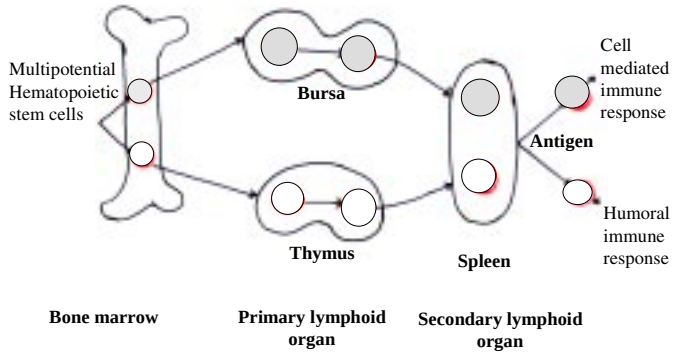

Fig. 2. Diagrammatic representation of development and migration of T-cells and B-cells.

of immunocompetent and peripheral blood cells, has received less attention in relation to the pineal gland (Fig. 2) The progenitors of hematopoietic cells in bone marrow exhibit continuous proliferation and differentiation (Haldar *et al*., 1992b) and are highly vulnerable to acute / chronic oxidative stress (Reiter *et al*., 1993; 1995.). Preliminary studies also suggest melatonin presence and synthesis in bone marrow (Conti and Maestroni, 1998;Tan *et al*., 1999; Vijayalaxmi *et al*., 2000). Further, peripheral blood and bone marrow cells are clinically important for diagnostic purposes.

Macrophages are widely distributed cell type and show heterogeneous antigenic and functional properties (Rutherford *et al*., 1993; Pirami *et al*., 1991). They play a central role in specific and unspecific immune response (Allen and Unanue, 1984; Adams and Hamilton, 1984) and also participate in regulation of haemopoiesis (Broxmeyer *et al*., 1989; Lee *et al*., 1988, Lee, 1991). Most data exists on the proliferation and differentiation of bone marrow cells under the influence of macrophage colony stimulating factor (M-CSF) and granulocyte macrophages colony stimulating factors (GM-CSF) on mice and rats (Keller *et al*., 1989; Krugluger *et al*., 1990; Willman *et al*., 1989). Vijayalaxhmi *et al*. (2000) reported that more than 50% of the melatonin synthesis occurs in the rat bone marrow. But, bone marrow, which is a remarkable immune marker (bone marrow macrophages), has been studied less in relation with the melatonin except by Haldar *et al*. (1992b). In our investigation, the interesting finding was the high counting of bone marrow lymphocytes after exogenous melatonin treatment to the squirrels and also an extremely high counting of peripheral blood lymphocytes as well as total leukocyte count, which was noted very low in the Px squirrels and was recovered to the control level when the Px squirrels were treated with melatonin.

Bone marrow is an important tissue in generation of immunocompetent and peripheral blood cells. The progenitors of hematopoietic cells in bone marrow exhibit continuous proliferation and differentiation and they are highly vulnerable to environmental oxidative insults, such as irradiation, chemotherapeutic compounds, oxidative stresses and other environmental pollutants. Tan and his coworkers (1999) identified a highly elevated level of pineal melatonin by using immunocytochemistry, radioimmunoassay, high performance liquid chromatography with electrochemical

detection and mass spectroscopy. In their finding the night time melatonin concentrations in the bone marrow of rats were roughly two orders of magnitude higher than those in peripheral blood. Important point is that bone marrow cells have a measurable N-acetyltransferase (NAT) activity but they have very low level of hydroxyindole-O-methoxyltransferase (HIOMT) activity. Moreover, melatonin level in the bone marrow followed the same pattern as in the serum i.e. bone marrow melatonin concentration showed a reasonably good correlation with circulating levels. This potential finding about the identification melatonin synthesis in the bone marrow was a very interesting one, which turned us towards the direction to find about the relationship between the bone marrow lymphocytes with that of the pineal melatonin. It is now well recognized that a main actor in the continuous interaction between the nervous and immune system is the pineal hormone melatonin. Hematopoiesis is apparently influenced by the action of the melatonin-induced immuno-opioid (MIIO), which is recognized as novel opioid cytokines on kappa-opioid receptors present on stromal bone marrow macrophages. Most interestingly, gamma–interferon (ã-INF) and colony stimulating factors (CSFs) may modulate the production of melatonin in the pineal gland. A hypothetical pineal-immune-hematopoietic network is therefore, taking a shape. From the immuno-pharmacological and ethical point of view, clinical studies on the effect of melatonin in combination with IL-1, IL-2 or other cytokines in viral diseases including human immunodeficiency virus infected patients and cancer patients are needed. Melatonin seems to play a crucial role in the homeostatic interactions between the brain and the immune hematopoietic system and deserve to be further studied to identify its therapeutic indications and its adverse effects.

In view of the variety of possible direct effects of melatonin on intracellular signaling (the existence of different type of melatonin receptors) and the fact that lymphocytes themselves may produce melatonin in response to certain stimuli, melatonin, in addition to its endocrine role, can be expected to have a physiological impact as a paracrine or even intracellular modulator within immune system. The melatonin receptors are present on the lymphoid organs as well as on lymphocytes suggest that melatonin plays a key role in immunomodulation (Calvo *et al*., 1995).

Melatonin and Rhythm in Bone Marrow Macrophages

Each vertebrate species possess a characteristic rhythm of daily activity, being either diurnal (when the mental and locomotor activity as rule are enhanced during the light period of the day while sleep or resting coincides with the period of darkness) or nocturnal (active during the darkness like mice, hamsters and rats).

In mice (Kuci *et al*., 1988) and rat (Haldar *et al*., 1992b) circadian control of immune system has been observed. The pineal gland is believed to regulate the circadian aspects of immune function (Kuci *et al*., 1988; Haldar *et al*., 1992b). On the

other hand product of immune system like lymphokines and monokines modulate the neuroendocrine function while various endocrine signals also affect the immune function. Neuroendocrine and lymphoid cells share a number of neurotransmitters, neuromodulatory substances supporting the existence of bi-directional and immune system (Fabris, 1994, Skwarlo –Sonta, 2002; Skwarlo –Sonta *et al*., 2003).

Generally, all thoroughly investigated immune parameters of man and vertebrate animals presents a circaannual and circadian pattern, including the number of circulating immunocompetent cells (leukocytes, blood lymphocyte and lymphocyte of bone marrow) and their products (interleukins) that are about two or three times greater at a peak compared with a trough (Maes *et al*., 1994). Circadian periodicity in the components of the immune system and immune function such as the levels of circulating immunoglobulins variation in serum complement proteins, or variability in the number of circulating lymphocytes has been reported. The GM-colonies were either tightly packed or loosely dispersed cell aggregates having more than 1000 cells. Pinealectomy (Px) reduced colony numbers significantly (~25%). The numbers of colonies for CFU-GM in the bone marrow cells of those Px squirrels which were subcutaneously injected with melatonin was highly significant (P<0.001) than the control and Px squirrels (Fig. 3). In the control group a clear rhythm in colony formation of granulocyte-macrophages was noted. It had a peak at 18.00 hrs with an average of 111 colonies and at 22.00 hrs less colonies (50.2 colonies) were noted. Pinealectomy of the squirrels almost obliterated the rhythm of proliferation of CFU-GM maintaining a significantly (P<0.001) low profile of colony of granulocyte macrophages with a free running pattern in comparison to the control group. Px squirrels receiving melatonin showed a high number of colonies for CFU-GM at an average of 130 colonies at 18.00 hrs and a minimum numbers of colonies at 10.00 hrs. The pinealectomized squirrels, receiving exogenous melatonin, clearly attained the rhythm of proliferation of CFU-GM, which was almost up to the level of control group of squirrels (Fig 3).

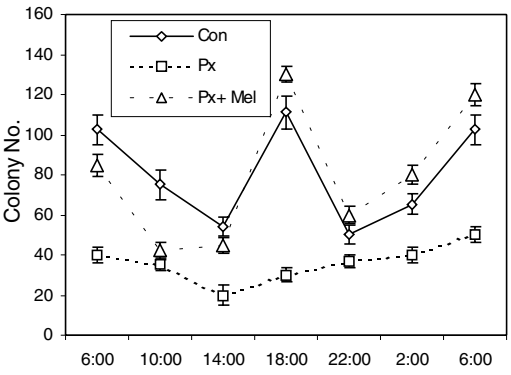

Fig. 3. Effect of exogenous melatonin on rhythmicity of Granulacyte Macrophages – Colony Forming Unit from bone narrow of Indian palm squirrel, *F. pennanti,* Mean ± SEM. Con = Control, Px-pinealectomized and Px + Mel = Pinealectomized + melatonin treatment.

From the literature we could locate that till date such study was performed only in laboratory rodent such as mice and rat (Kuci *et al*., 1988; Haldar *et al*., 1992b). The regulatory role of pineal melatonin on proliferation of CFU-GM can be clearly judged by observing the data of the pinealectomized squirrels. Px during

morning hrs was noted ineffective for physiological function in these squirrels as well as in rat (Haldar *et al*., 1992b) for rhythm of CFU-GM therefore, we performed pinealectomy during afternoon, which is known for its physiological effect in this rodent (Haldar *et al*., 1992b). Melatonin treatment to the Px squirrels restored the rhythm of proliferation up to the complete control level unlike rats (Haldar *et al*., 1992b). These observations strongly suggest that the pineal gland and its hormone melatonin is having an effective modulatory role on immune system of this squirrel which was earlier reviewed by Mcgillis *et al*. (1983), Maestroni *et al*. (1998); Conti *et al*. (2000) in mice and rats.

Melatonin, substitution not only restored the CFU-GM proliferation in the Px animal, but also restored its rhythmic pattern. Evidences have demonstrated that the various circadian rhythm of vertebrate species including humans, are synchronized by the administration of exogenous melatonin as the suprachiasmatic nuclei (SCN) have mel 1a receptor and hence, directly effected by melatonin (Cassone, 1990).

As far as hematopoiesis is concerned, melatonin has been shown to possess a stimulatory effect on mouse GFU-GM (Kuci *et al*., 1988) and rats (Haldar *et al*., 1992a). However, melatonin has been shown to protect the blood forming system of mice transplanted with Lesis lung carcinoma and treated with Cyclophosphamide or Etoposide (Maestroni *et al*., 1994a,b). *In vitro* melatonin rescued hematopoietic progenitors from apoptosis induced by Etoposide or Carbolated in rats. Consistently, melatonin at both physiological and pharmacological concentrations brought back the rhythm of CFU-GM (Haldar *et al*., 1992a, 1992b) in rats. It increased the number of granulocytes/macrophage colony forming units (GM-CFU), when added directly to bone marrow culture, but only in the presence of sub optimal concentration of CSF (Maestroni *et al*., 1994a,b). The rhythm of CFU-GM in bone marrow cell in control squirrel appears to be an ultradian type having duration of approximately 8 hrs. The numbers of colonies of 6.00 hrs and 18.00 hrs (high peak

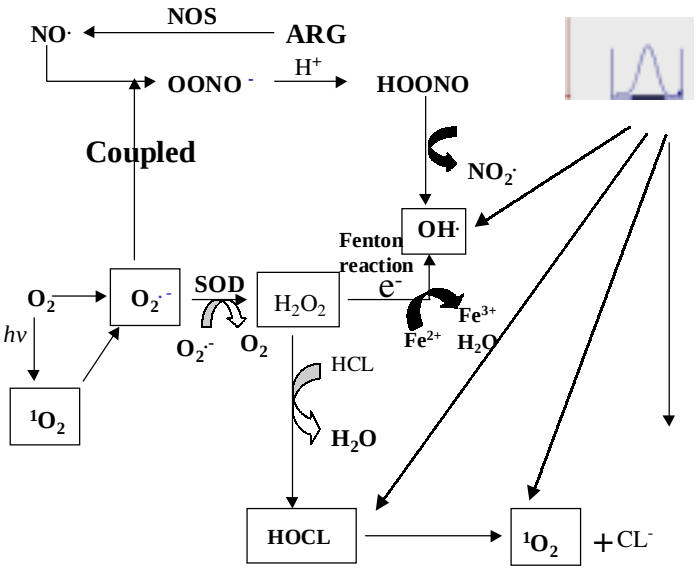

Fig. 4. Free radical generation and action of melatonin.

values) were significantly different from the number recorded at other hours of CFU-GM proliferation.

Afternoon injection of melatonin was effective in restoration of various physiological, hormonal and endocrine factors of this squirrel (Haldar *et al*., 1992b). Similarly, we found that evening injection of melatonin was highly effective not only in restoring colony numbers but also the rhythm pattern with complete efficiency. The support for above statement comes from the data of Px and Px squirrel receiving the exogenous melatonin group. Though, the above effect is independent of the time of the surgery but our study is in good agreement with the reported periodicity in component of the immune system earlier by McGillis and his coworkers (1983) in mice. The peaking of the CFU-GM proliferation noted by us is similar to that of rat reported by Haldar *et al*., (1992b) but is different to mice reported by Kuci *et al*., (1988), which could be due to different species used. However, the general agreement in the literature that the time of optimal immunologic functions occurs shortly before or after the onset of light in rodent i. e. at 6.00 hrs (McGillis *et al*., 1983). The result from the present study demonstrate that the communication between the neuro-endocrine and immune system could be mediated by signals from the pineal gland, which also has been observed for the other peptides such as β-endorphin, enkephalins, interleukins and thymosins (Lissoni *et al*., 1986; Ader *et al*., 1990; Gupta, 1990).

Pinealectomy and Immunity

Melatonin in general affects immune system via lymphoproliferation in lymphoid organs (spleen and thymus) and lymph nodes. Studies have not definitely determined to date a relationship between bone marrow cells and peripheral blood cells with that of melatonin administration in pinealectomized rodents. We observed low hematological parameters in pinealectomized (Px) squirrels, i. e. having low TLC and LC (count and %) of peripheral blood and bone marrow and also decreased number of cells in histological section of thymus and spleen along with high peripheral testosterone. Our observation suggest that exogenous melatonin was able to restore the immune status that was depressed by high testosterone in sham control group led us to investigate its role in Px squirrels (Haldar and Singh, 2001). When Px squirrels were treated with melatonin 25mg/100g body mass they showed restoration of all the hematological parameters beyond normal levels. Removal of pineal gland does not affect the individual's hormonal level and immune status much, but keeps them reproductively active for a very long period, thereby, leading to the low immunity even under unfavorable winter and renders them more susceptible to disease and death. Reproductive phase dependent variation / seasonal changes in lymphatic organs were presumed to reflect changing organ/cell function. The cell density observed in thymus and spleen after Px (low) and melatonin injection (restored) provides an obvious link among these organs to seasonal change with pineal and

reproductive functions. It also supported by many early hypothesis suggesting that the lymphoid organs regulated or influenced by breeding phase (Nelson *et al*., 1998), when the peripheral melatonin is low.

Our Px results suggested increased (~1.5 - 1.52 ng/ml) gonadal steroid, which might have suppressed immune status of Px squirrels. Injection of melatonin to Px squirrels restored the immune status up to control level. Other studies (*in vivo* and *in vitro*) on thymus and spleen in relation with the melatonin treatment suggested that melatonin inhibited apoptosis of thymocytes and splenocytes (Sainz *et al*., 1995) and might be protecting the cells from DNA damage (Tan *et al*., 1993b).

Melatonin receptor involvement

The literature suggests that melatonin enhanced immune function in most normal cases where the basal level of melatonin is low (Nelson and Demas, 1997). The complexity to our understanding on the physiological role of melatonin towards its immunomodulatory and hematopoietic functions in vertebrates, including humans is still matter of debate, however reports suggest that melatonin acts through specific receptors located on the plasma (Calvo *et al*., 1995) and nuclear membrane (Garcia-Maurino *et al*., 1998) of lymphocytes and monocytes (Barjavel *et al*., 1998), in bone marrow T- helper cells (Maestroni, 1995), in $CD8^+$ -T and B-cells (Guerrero *et al*.,1996; Garcia-Maurino *et al*., 1997a,) and purified cell nuclei from the spleen and thymus of rat (Rafii-El-Idrissi *et al*., 1998). Melatonin may interact with the immune system via other hormones like declining secretion of steroids, PRL, and opiatergic i.e. via opioid receptor mediated (Poon and Pang, 1992; Calvo *et al*., 1995 Maestroni *et al*., 1988). *In vitro* studies are also valuable because they remove the confounding influence of concurrent circulating hormone. Previous studies of in vitro melatonin treatment to mice and rats reported enhanced immune function (Fraschini *et al*., 1990, Guerrero and Reiter, 1992; Atre and Blumenthal, 1998). Specifically, melatonin enhances IL-2 and interferon ã (Garcia-Maurino *et al*., 1997a, 1998), but, some other studies on in vitro melatonin treatment report inhibitory as well as (Persengiev and Kyurkchiev, 1993; Di Stefano and Paulesu, 1994; Maestroni *et al*., 1987b) on immune function.

We have also noted the down regulation of percent expression of receptor for melatonin (Mel 1a R) in melatonin treated lymphoid organs were noted suggesting a direct action of melatonin on lymphatic cells of this squirrel. The melatonin receptor Mel 1a is a member of the super family of guanine nucleotide-binding regulatory protein i.e. (G-protein) – coupled receptor (Brydon *et al*., 1999; Roka *et al*., 1999). Signaling through melatonin receptors inhibits adenylate cyclase and stimulates phospholipase Câ upon activation of pertusis toxin (PTX) – sensitive G-proteins (Brydon *et al*., 1999; Roka *et al*., 1999). It is expressed as 37 kD protein localized on to splenocytes, thymocytes and bone marrow lymphocytes and implicated in controlling cellular growth in response to melatonin (Shiu *et al*., 2000). Therefore, it

can be suggested that melatonin may act both directly and indirectly via other endocrine organ to affect immune function. Melatonin can act directly on high affinity receptors located on bone marrow lymphocytes to increase immune function (Pang and Pang, 1992; Calvo *et al*., 1995). The result of the present study support the hypothesis that melatonin is acting, at least in part, directly on lymphocyte proliferation. It is possible that melatonin binds to receptors and increased the general proliferative ability of these cells, leading to enhanced cell mediated immunity. Consistent with this idea, high–affinity melatonin receptors have been localized on circulating lymphocytes (Pang and Pang, 1992; Calvo *et al*., 1995).

Our data also suggest that the neuroendocrine signal i.e. melatonin parallel the circadian rhythmicity of immune parameters, which could be of great importance in adaptive significance of the squirrel, which is a seasonal breeder. T-helper cells (T_H) hemopoietic protection is involved in the release of granulocyte macrophages colony stimulating factor (GM-CFU) from bone marrow upon stimulation by a T_H cell factor induced by melatonin (Maestroni *et al*., 1994a,b). Both activated lymph nodes, T_H cells and bone marrow T_H cells releases novel opioid cytokines, which was named as melatonin-induced-immuno-opioid (MIIO) (Maestron *et al*., 1996; Maestroni, 2000). MIIO is a decapeptide, released by $CD4^+$ lymphocytes upon stimulation and belong to a new family of endogenous kappa-opioid agonist (Maestroni and Conti, 1990). Hematopoiesis is influenced by the action of MIIO on kappa-opioid receptors present on stromal bone marrow macrophages (Maestroni, 1998, Meastroni *et al*., 1999). Further, exogenous melatonin may optimize the immune response by sustaining T_H cells and macrophage functions and production of cytokines (MIO, IL-6, IL-1) may also affect hematopoiesis. Our Px data partially reflects to the physiological requirement of melatonin for regulation of hematopoiesis. The proper function of above might be crucial in the adaptive response of the organism to environmental demands and thus in the maintenance of health.

Immunity and free radicals vs melatonin

The pineal gland and melatonin were initially identified as the interface between the prevailing environmental photoperiod and seasonal reproductive capability in photoperiodic mammals. Subsequently, melatonin was linked to phenomena such as sleep and circadian rhythms. More recently functions of melatonin include effects on immune system and as an oncostatic agent. Finally in 1993, it was reported that melatonin is also a free radical scavenger and an antioxidant (Tan *et al*., 1993).

Further, melatonin can act on lymphoid organs following receptor mediated and non-receptor mediated pathway. Among receptor-mediated pathway firstly it acts through protein kinase pathway (signal transduction) and by activating cAMP gives cellular responses. Secondly, it acts through Calmodulin Ca^+ channel pathways and gives

physiological responses by activating affector protein. Melatonin can directly acts on lymphoid organ via orphan nuclear receptor family RZR/ROR responsible for synthesis of 5-lypoxygenase genes. Besides its obvious interaction with membrane and nuclear receptors in immune cells melatonin has non-receptor-mediated action in all systems, including immune system. These effects are a consequence of melatonin's ability to directly scavenge free radicals by donating an $\bar{e}$ this system.

Molecules that have neutralize, detoxify, or scavenge the ·OH play an important role protecting against oxidative stress. Molecules that have capability of detoxifying radical species are referred to as antioxidants. Their role allows them to prevent macromolecular damages and over dysfunction that results from the mutilation. The ability of melatonin to directly scavenge the ·OH was proven using a combination of spin trapping methodologies and electron spin resonance (ESR) spectroscopy (Matuszek *et al*., 1997; Tan *et al*., 1993). Besides ESR, pulse radiolysis and additional indirect methods showed that melatonin neutralized the ·OH with a high degree of efficiency (Mahal *et al*., 1999; Roberts *et al*., 1998; Stasica *et al*., 1998). The rate constant for the scavenging of the ·OH by melatonin is calculated to be on the order of 2.7×10^{10} $M^{-1}s^{-1}$ (Matuszek *et al*., 1997, Poeggeler *et al*., 1996). Additionally, melatonin may also act synergistically with other antioxidant in the detoxification of the ·OH and other reactive intermediates (Mahal *et al*., 1999; Poeggeler *et al*., 1996).

Direct antioxidative action of melatonin

Most recently, a product of the interaction of melatonin with two ·OH was noted. This product is a novel metabolite, Cyclic 3-hydroxymelatonin (3-OHM) (Tan *et al*., 1998). This Cyclic 3-hydroxymelatonin (3-OHM) is excreted in urine of mammals. Since, the amount of Cyclic (3-OHM) varies with the level of oxidative stress, the animal has recently experienced, the metabolite may be a valuable biomarker of *in vivo* ·OH generation, and it may be useful as a clinical index of the oxidant status of an individual or the presence of a free radical disease. Cyclic 3-OHM is the signature molecule that results when melatonin scavenges two ·OH. Although the detoxification of the ·OH itself is an important accomplishment, but melatonin direct scavenging actions do not end with this action. Melatonin also repeatedly neutralizes its precursor, hydrogen peroxide (H_2O_2) and other oxidants including singlet oxygen ($^1O_2$) nitric oxide and (NO·), and the product of the interaction of super-oxide anion radical (O^-_2) and NO·, namely, peroxynitrite anion ($ONOO^-$) and or its other metabolite which exhibit very high toxicity. Overall melatonin is certainly capable of directly neutralizing a variety of free radicals and or their reactive intermediates and thereby reducing the macromolecular destruction.

Indirect antioxidative action of melatonin

Antioxidants can function as direct scavengers of free radicals and reactive oxygen species or they can act indirectly

to metabolize free radicals and their intermediates into harmless products. A number of indirect antioxidants are enzymes that remove toxic molecules either before they damage the cell or prevent more toxic agents from being formed. Classic antioxidative enzymes include the superoxide dismutase (SOD), subspecies of which are located throughout the cell, and the glutathione (GSH) peroxidases (GPx), GSH reductase (GRd), and catalase (CAT). The GPx and CAT catalytically remove and H_2O_2 and lipid hydroperoxides from the cell, thereby reducing the generation of the ·OH.

Our study states high lipidperoxidation (LPO) in the bone marrow, spleen as well as thymus of control group of squirrels than those squirrels, which were treated with the exogenous melatonin. Further, high level of lipidperoxidation was found in the pinealectomized group of the squirrels which was restored up to the control level in pinealectomized squirrels treated with the similar concentration of the melatonin as that of the control groups.

Burgeoning knowledge of the seasonal activity patterns of the different parts of the immune system will enable us to devise more precise immunological experiments; these may include challenging various parts of the immune system at different times of the year. Further, species-specific studies are needed to investigate natural patterns of the immune function and to test whether alterations in these rhythms are caused by breeding or by difference in the challenge to the immune system at certain times of the year (Fig.4).

Conclusion

The breeding period itself may cause physiological stress, which can *per se* affect immune function. This relation also establishes the "immunocompetence handicap" hypothesis between immunity and reproduction to help the individual for survival. Therefore, melatonin is required for enhancing immune function in bone marrow of seasonal breeder to cope with seasonal stressors that would otherwise compromise immune functions during sexually inactive period/winter. However, we are still far from a complete understanding of the mechanism underlying the immunological and hematopoietic action of melatonin. This fact calls for the further study to understand the role of melatonin in hematopoiesis and indicates that, melatonin may be considered as a potential therapeutic agent and an important endogenous immunomodulator.

In summary, it may be suggested that melatonin may be part of an integrative system to coordinate reproductive, immunologic and other physiological processes to cope successfully with energetic stressors. Further, this brief review also emphasizes that melatonin affects immune function, influencing both humoral and cell-mediated immunity.

Literature cited

Adams, D. O. and Hamilton, T. A. 1984. The cell biology of macrophage activation. *Ann. Rev. Immunol.*, **2:** 283-318.

Ader, R., Felten, D. and Cohen, N. 1990. Interaction between the brain and the immune system. *Ann. Rev. Pharmacol. Toxicol.,* **30:** 561-602.

Allen, P. M. and Unanue, E. 1984. Differential requirements for antigen processing by macrophages for Lysozyme-specific T cell hybridomas. *J. Immunol*., **132:** 1077-1079.

Arendt, J. 1988. Melatonin. *Clin. Endocrinol*. (Oxford)., **29:** 205.

Arendt, J. 1995. *Melatonin and the Mammalian Pineal Gland*. Chapman and Hall, London.

Armstrong, S. M., and Redman, J. R. 1991. Melatonin: A chronobiotic with anti-aging properties. *Med. Hypothesis.,* **34:** 300.

Atre, D. and Blumenthel, E. J. 1998. Melatonin: Immune modulation of spleen cells in young, middle-aged, and senescent mice. *Mech. Aging Dev.,* **103:** 255- 268.

Bentley, G. E, Demas, G. E, Nelson, R. J. and Ball, G. F. 1998. Melatonin, immunity and cost of reproductive state in male European starlings. *Proc. R. Soc. Lond. B. Biol. Sci*., **265**: 1191-1205.

Barjavel, M. J., Mamdouh, Z. and Raghbate, N. 1998. Differential expression of the melatonin receptors in human monocytes. *J. Immunol.,* **160:** 1191-1197.

Broxmeyer, H. E., Sherry, B. and Lu, L. 1989. Myelopoietic enhancing effects of murine macrophage inflammatory protein 1 and 2 on colony formation *in vitro* by murine and human bone-marrow granulocyte macrophage progenitor cells, *J. Exp. Med.,* **170:** 1583-1594.

Brydon, L., Roka, F., Petit, L., Copper, P., Tissot, M., Barrett, P., Morgan, P. J., Nanoff, C., Strosberg, A. D. and Jockers, R. 1999. Dual signaling of human Mel 1 a melatonin receptor via G (12), G (i3) and G (G/11) protein. *Mol. Endocrinol.*, **13:** 2025-2038.

Bubenik, G. A. 2002. Gastrointestinal melatonin: A Cinderella story of melatonin research. In: *Treatise on Pineal Gland and Melatonin*, (Eds.) C Haldar, M Singaravel, SK Maitra, (Eds.) Science Publisher, Inc. Enfield (NH), USA.

Calvo, J. R., Rafii-El-Idrissi, M., Pozo, D. and Guerrero, G. M. 1995. Immunomodulatory role of melatonin: specific binding sites in human and rodent lymphoid cells. *J. Pineal Res.,* **18:** 119-126.

Cassone, V. 1990. Effect of melatonin on vertebrate circadian systems. *Trend. Neurosci.,* **13:** 475-465.

Conti, A., Conconi, S., Retenis, E., Skwarlo-Sonta, K., Markowska, M., and Maestroni, G. J. M. 2000. Evidence for melatonin synthesis in mouse and human bone marrow cells. *J. Pineal Res*., **28:** 193-202.

Conti, A. and Maestroni, G. J. M. 1998. Endogenous melatonin in serum and bone marrow of inbred and outbred mice, *In: Thymus and Pineal Gland in Neuroimmunoendocrinology*. Swieradow Zdrog, 22B, abstract.

Demas, G. E. and Nelson, R. J. 1996. The effect of photoperiod and temperature on immune function of adult male deer mice (*Peromyscus maniculatus*). *J. Biol. Rhythms*., **11:** 94-102.

Di Stefano, A. and Paulesu, L. 1994. Inhibitory effect of melatonin on production of IFN- ã of TNF- á in peripheral blood mononuclear cells of some blood donors. *J. Pineal Res.,* **17**: 164-169.

Fraschini, F., Scanglione, F., Demartini, G., Lucini, V. and Sacerdote. 1990. Melatonin action on immune response In: *Advances in Pineal Research.* RJ Reiter, A Lucaszyk (Eds) John Libbey, London pp 225 –233.

Garcia-Maurino, S., Gonzales-Haba, M. G and Calvo, J. R. 1998. Involvement of the immune binding sites for melatonin in the regulation of IL-2 and IL-6 production in human blood monolayer cells. *J. Neuroimmunol.,* **92:** 76-84.

Garcia-Maurino, S., Gonzalez-Haba, M. G., Calvo, J. R., Rafii-El-Idrissi, M., Sanchez-Margalet, V., Goberna, R. and Guerrero, J. M. 1997a. Melatonin enhances IL-2, IL-6, and IFN-gamma production by human circulating CD4+ cells: a possible nuclear receptor – mediated mechanism involving T helper type I lymphocytes and monocytes. *J. Immunol.,* **159:** 574-581.

Giordana, M., Vermullen, M. and Palermo, M. S. 1993. Seasonal variations in antibody, cellular

toxicity regulation by melatonin. *FASEB J.,* **7:** 1052-1054.

Guerrero, J. M., Garcia-Mourino, S., Gil-Haba, M., Rafii-El-Idrissi, M., Pozo, D., Garcia-Perganeda, A. and Calvo, J. R. 1996. Mechanism of action on the human immune system: Membrane receptors versus nuclear receptors. In: *Therapeutic Potential of the Pineal Hormone Melatonin.* Maestroni, G.J.M., Conti, A., Reiter, R.J. (Eds.); Karger, Basel, pp. 43-52.

Guerrero, J. M. and Reiter, R. J. 1992.A brief survey of pineal gland-immune system interrelationships. *Endocrinol. Res.,* **18:** 91-113.

Gupta, D. 1990. The pineal gland: its immunomodulatory role. In: *Advances in Pineal Research,* vol. 4, Reiter RJ, Lukaszyk A. (Eds.) John Libbey, London. 265-285.

Haldar, C., Haussler, D. and Gupta, D. 1992a. Response of colony forming units for granulocyte macrophages (CFU-GM) of intact and pinealectomized rat bone marrow cells to murine recombinant interleukin-3 (rIL-3), recombinant granulocyte macrophage colony stimulating factor (rGM-CSF) and human recombinant erythropoietin (rEPO). *Prog. Brain Res*., **91 :** 323-325.

Haldar, C., Haussler, D. and Gupta, D. 1992b. Effect of the pineal gland on circadian rhythmicity of colony forming units for granulocytes and macrophages (CFU-GM) from rat bone marrow cell cultures. *J. Pineal Res.,* **12:** 79-83.

Haldar, C. and Singh, R. 2001. Pineal modulation of thymus and immune function in a seasonally breeding rodent, *F. pennanti. J. Exp. Zool.,* **286**: 90-98.

Haldar, C. 1996. Patterns of plasma melatonin in Indian tropical palm squirrel, *F. pennanti*. In: *Role of pineal gland: its molecular signals,* C. Haldar, (Ed.) HPCL, New Delhi, pp. 95-117.

Hoffman, R. A, and Reiter, R. J. 1965. Pineal gland: Influence on gonads of male hamsters. *Science.,* **148:** 1609-1611.

Keller, R., Joller, P. and Keist, R. 1989. Surface phenotype of rat bone marrow- derived mononuclear phagocytes. *Cell Immunol*., **120:** 277-285.

Kliger, C. A., Gehad, A. E., Hulet, R. M., Roush, W. B., Lillehoj, H. S. and Mashaly, M. M. 2000. Effect of photoperiod and melatonin on lymphocyte activities in male broiler chicken. *Poul. Sci*., **79:** 18-25.

Krugluger, W., Gessl, A., Boltz-Nitulescu, G. and Forster, O. 1990. Lectin binding of rat bone marrow cells during colony stimulating factor type 1 induced differentiation: soybean agglutinin as a marker of mature rat macrophages. *J. Leucoc. Biol.,* **48:** 541-548.

Kuci, S., Becker, J., Viet, G., Handgretinger, R., Attanasio, A., Bruchelt, G., Treuner, J., Niethammer, D. and Gupta, D. 1988. Circadian variation in the immunomodulatory role of the pineal gland, *Neuroendocrinol. Lett*., **10:** 65-79.

Lee, S. H., Crocker, R. R and Westaby, S. 1988. Isolation and Immunocytolocalization of human bone marrow stromal macrophages in hemopoietic clusters, *J. Exp. Med.,* **168:** 1193-1208.

Lee, S. H. 1991. Phenotypic analysis of human bone marrow macrophages, *Blood Cells*, **17:** 45-54.

Lissoni, P., Marelli, O., Mauri, R., Resentini, M., Franco P., Esposti, D., Esposti, G., Fraschini, F., Halberg, F., Sothern, R. and Cornelissen, G. 1986. Ultradian chronomodulation by melatonin of a placebo effect upon human killer cell activity, *Chronobiologia.* **13:** 339-343.

Maes, M., Stevens, W., Scharpe, S., Bosmans, E., De Meyer, F., Do Hondf, P., Peeters, D., Thompson, P., Cosyns, P, De-clerk, L., Neels, H., Wauters, A. and Cooreman, W. 1994. Seasonal variation in peripheral blood leukocyte subsets and in serum interleukin-6 and soluble interleukin-2 and 6 receptor concentrations in normal volunteers. *Experientia.,* **50:** 821-829.

Maestroni, G. J. M. and Conti, A., Lissoni, A. 1994a. Hematopoietic rescue via T- cell dependent

endogenous GM– CSF by the pineal neurohormone melatonin in tumor bearing mice. *Cancer Res*., **54:** 2429-2432.

Maestroni, G. J. M, Conti,A, and Lissoni, P. 1994b. Colony stimulating activity and hematopoietic rescue from cancer chemotherapy compounds are induced by melatonin via endogenous interleukin-4. *Cancer Res*., **54:** 4740-4743.

Maestroni, G. J. M., Conti, A. and Pierpaoli, W. 1987b. Role of pineal gland in immunity. II. Melatonin enhances the antibody response via an opiatergic mechanism. *Clin. Exp. Immunol.,* **68:** 384-391.

Maestroni, G. J. M., Conti, A. and Pierpaoli, W. 1988. Role of the pineal gland in immunity. III, Melatonin antagonizes the immunosuppressive effect of acute stress via an opiatergic mechanism. *Immunology*., **63:** 465-469.

Maestroni, G. J. M., Conti, A. and Pierpaoli, W. 1998. Melatonin stress and the immune system. In : *Pineal Research Review,* RJ Reiter, (Ed.), New York, Liss, 203 –223.

Maestroni, G. J. M. and Conti, A. 1990. The pineal neurohormone melatonin stimulate activated CD4+, Thy – 1+ cells to release opioid agonist(s) with immunoenhancing and anti stress properties. *J. Neuroimmunol.,* **28:** 167 – 176.

Maestroni, G. J. M., Hertens, E. and Galli, P. 1996. Melatonin induced T-helper cell hematopoietic cytokines resembling both interleukin-4 and dynorphin. *J. Pineal Res*., **21**: 131-139.

Maestroni, G. J. M., Zammaretti, F. and Pedrinis, E. 1999. Hematopooietic effect of melatonin. Involvement of k-1 opioid receptor on bone marrow macrophages and interleukin-1. *J. Pineal Res.,* **27:** 145-153.

Maestroni, G. J. M. 1995. T helper 2 lymphocytes as a peripheral target of melatonin. *J. Pineal Res.*, **18:** 84–89.

Maestroni, G. J. M. 2000. MLT and the Immune Hematopoietic system, In: *Melatonin after four decades: An assessment of its potential* James Olcere, (Ed.) *Advances in Experimental Medicine and Biology*., **460:** 395-405.

Maestroni, G. J. M. 1998. The photoperiod transducer melatonin and the immune-hematopoietic system. *J. Photoch. Photobiol*., **43:** 186-192.

Mahal, H. S., Sharma, H. S. and Mukherjee, T. 1999. Antioxidant properties of melatonin: a pulse radiolysis study. *Free Radic. Biol. Med.,* **26:** 557-565.

Matuszek,A., Reszka, K. J. and Chignell, C. F. 1997. Reaction of melatonin and related indoles with hydroxyl radicals: ESR and spin trapping investigations. *Free Radic. Biol. Med*., **23:** 367-372.

Mcgillis, J. P., Hall N. R. and Goldstein. 1983. Circadian rhythm of thymosin a1 in normal and thymectomized mice. *J. Immunol*., **131:** 148-151.

Nelson, R. J., Demas, G. E., Klein, S. L. and Kriegsfeld, L. J. (Eds) 2002. *Seasonal patterns of stress, immune function and, disesse*. Cambridge Univ. Press. New York.

Nelson, R. J., Demas, G. E. and Klein, S. L. 1998. Photoperiodic mediation of seasonal breeding and immune function in rodents: A multi–factorial approach. *Am. Zool.,* **38:** 226 – 237.

Nelson, R. J. and Demas, G. E. 1996. Seasonal changes in immune function. *Q. Rev. Biol*., **71:** 511-548.

Nelson, R. J. and Demas, G. E. 1997. Role of melatonin in mediating seasonal energetic and immunologic adaptations. *Brain Res. Bull.,* **44:** 423-431.

Nir, I. 1995. Biorhythms and the biological clock involvement of melatonin and the pineal gland in life and disease. *Biomedical. Envtal. Sci.,* **8:** 90.

Pang, C. S. and Pang, S. F. 1992. High affinity specific binding of 2[^{125}I] iodomelatonin by spleen membrane preparations of chicken. *J. Pineal Res*., **12:** 167-173.

Persengiev, S. P. and Kyurkchiev, S. 1993. Selective effect of melatonin on the proliferation of lymphoid cells. *Int. J. Biochem.,* **24:** 441-444.

Pioli, C., Caroleo, M. C., Nistico. and G., Doria, G. 1993. Melatonin increases antigen presentation and amplifies specific and non-specific signals for T-cell proliferation. *Int. J. Immunopharmacol.*, **15:** 463-468.

Pirami, L., Stockinger, B., Corradin, S. B. 1991. Mouse macrophage clone immortalized by retroviruses are functionally heterogenous, *Proc. Natl. Acad. Sci.*, U.S.A., **88:** 7543-7547.

Poeggeler, B., Reiter, R. J., Hardeland, R., Tan, D-X and Barlow-Walden, L. R. 1996. Melatonin and structurally related endogenous indoles act as potent electron donors and radical scavengers *in vitro. Redox Reports.,* **2:** 179-184.

Poon, A. M. S., Liu, Z. M., Tang, F. and Pang, S. F. 1994. Cortisol decreases 2[^{125}I] iodomelatonin binding sites in the duck thymus. *Eur. J. Endocrinol.,* **130:** 320-324.

Poon, A. M. S. and Pang, S. F. 1992. 2[125I] iodomelatonin binding sites in spleens of guinea pigs. *Life Sci.,* **50:** 1719-1726.

Poon, A. M., Liu, Z. M., Tang, F. and Pang, S. F. 1994. Evidence for a direct action of melatonin on the immune system. *Biol. Signals,* **3:** 107-117.

Rafii-El-Idrissi, M., Calvo, J. R. and Harmouch, A. 1998. Specific binding of melatonin by purified cell nuclei from spleen and thymus of the rat. *J. Neuroimmunol.*, **86:** 190-197.

Reiter, R. J., Melchiorri, D., Sewerynek, E., Poggeler, B., Barlow –Walden, L., Chuang, J. I., Ortij, G, G., and acuna-Castroviejo, D. 1995. A review of the evidence supporting melatonin's role as an antioxident. *J. Pineal. Res.,* **18:** 1 – 11.

Reiter, R. J., Poeggler, B., Tan, D. X., Chen, L. D. and Manchester, L. C. 1993. Antioxidant capacity of melatonin: A novel action not requiring a receptor. *Neuroendocrinol. Lett.*, **15:** 103.

Reiter, R. J. 1991. Pineal melatonin: cell biology of its synthesis and its physiological interactions. *Endocr. Rev.,* **12:** 151-180.

Reiter R J. 1982. The mammalian pineal gland. Structure and function. *Am. J. Anat.,* **162:** 287.

Roberts, J. E., Hu, D. N. and Wishart, J. E. 1998. Pulse radiolysis studies of melatonin and 6-chloromelatonin. *J. Photochem. Photobiol., B.,* **42:** 125-132.

Roka, F., Brydon, L, Waldhoer, M., Strosberg, A. D., Freissmuth, M., Jockers, R., and Nanoff, C. 1999. Tight association of the human Mel (1a)-melatonin receptor and G (i): precoupling and constitutive activity. *Mol. Pharmocol.*, **56:** 1014-1024.

Rutherford, M. S., Wittsell, A. and Schook, L. B. 1993. Mechanism generating functionally heterogenous macrophages: Chaos revisted. *J. Leucoc. Biol.*, **53:** 602-618.

Sainz R M, Mao J C, Uria H, Kotler M, Antolin F, Roadrignez C. and Menadez-Palacz A Z. 1995. The pineal neurohormone melatonin prevents *in vivo* and *in vitro* apoptosis of thymocytes. *J. Pineal Res.*, **19:** 178-188.

Shiu, S.Y., Li, L, Siu, S.W, Xi, S. C., Fong, S.W. and Pang, S. F. 2000. Biological basis and possible physiological implication of melatonin recepotor mediated signaling in rat epididymis. *Biol. Signal Receptors.*, **2:** 172-187.

Sharma S. 2002. Physiological role of melatonin in control of circadian rhythms of various hormones in Indian palm squirrel, *Funambulus pennanti*. Ph.D. thesis, Banaras Hindu University, Varanasi, India.

SkwarloSonta, K., Majewski, P., Markowska, M., Olap, R. and Olazanska, B. 2003. Bidirectional communication between the pineal galnd and immune system. *Can. J. Physiol. Pharmacol.* **81:** 342-349.

Skwarlo-Sonta, K. 2002. Melatonin in immunity: a comparative aspect. *Neuroendocrinol. Lett.* , **23** (Suppl. 1)**:** 61-66.

Stasica, P., Ulanski, P., Rosiak, and J. M. 1998. Melatonin as a hydroxyl radical scavenger, *J. Pineal Res*., **26:** 65-66.

Tan D. X., Chen, L. D., Poeggeler, B., Manchester, L. C. and Reiter, R. J. 1993. Melatonin a potent endogenous hydroxyl radical scavenger. *Endocrinol. J*., **1:** 57-61.

Tan, D. X., Manchester, L. C., Reiter, R. J., Qi, W. B., Zhang, M., Weintraub, S. T., Cabrera, J., Sainz, R. M. and Mayo, J. C. 1999. Identification of highly elevated level of melatonin in bone marrow: its origin and significance. *Biochimica. Biophysica. Acta.,* 206–214.

Tan, D. X., Manchester, L. C, Reiter, R. J., Plummer, B. F., Hardies, L. J., Weintraub, S. T., Vijayalaxmi and Shepherd, A. M. M. 1998. A novel melatonin metabolite cyclic 3-hydroxymelatonin: a biomarker of *in vivo* hydroxyl radical generation. *Biochem. Biophys. Res. Commun*., **253:** 614-620.

Tan, D. X., Poeggeler, B. and Reiter, R .J. 1993b. The pineal melatonin inhibits DNA adduct formation induced by the chemical carcinogen safrole *in vivo*. *Cancer Lett.,* **70:** 65-71.

Vijayalaxmi, Meltz, M. L., Reiter, R. J., Herman, T. S. 2000. Melatonin and protection from genetic damage in blood and bone marrow element: whole body irradiation studies in mice. *J. Pineal Res*., **27:** 221-225.

Willmann, C. L., Stewart, C. C., Miller, V., Yi, L.-L., Tomasi, T. B. 1989. Regulation of MHC Class II gene expression in macrophages by hematopoietic colony stimulating factors (CSF). *J. Exp. Med.,* **170:** 1559-1567.