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The role of melatonin in immuno-enhancement: potential application in cancer

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Summary

Melatonin, a neurohormone produced mainly by the pineal gland, is a modulator of haemopoiesis and of immune cell production and function, both *in vivo* and *in vitro*. Physiologically, melatonin is associated with T-helper 1 (Th1) cytokines, and its administration favours Th1 priming. In both normal and leukaemic mice, melatonin administration results in quantitative and functional enhancement of natural killer (NK) cells, whose role is to mediate defenses against virus-infected and cancer cells. Melatonin appears to regulate cell dynamics, including the proliferative and maturational stages of virtually all haemopoietic and immune cells lineages involved in host defense – not only NK cells but also T and B lymphocytes, granulocytes and monocytes – in both bone marrow and tissues. In particular, melatonin is a powerful antiapoptotic signal promoting the survival of normal granulocytes and B lymphocytes. In mice bearing mid-stage leukaemia, daily administration of melatonin results in a survival index of 30–40% *vs*. 0% in untreated mice. Thus, melatonin seems to have a fundamental role as a system regulator in haemopoiesis and immuno-enhancement, appears to be closely involved in several fundamental aspects of host defense and has the potential to be useful as an adjuvant tumour immunotherapeutic agent.

Keywords
cancer, cytokines, haemopeiesis, immunotherapy, melatonin, NK cells, T-helper cells

Introduction

Melatonin is a neuroimmunomodulator produced by the pineal gland, as well as by retina, gut and immunocompetent cells including both bone marrow cells (Conti *et al*. 2000) and lymphocytes (Carrillo-Vico *et al*. 2005). It is well-established as a chronomodulator of biological systems. Melatonin is conserved throughout evolution and acts to synchronize higher vertebrates with the light–dark cycle. Circulating melatonin, which is derived exclusively from the pineal gland, follows a circadian rhythm, being at maximal levels during

the dark period of the 24-h cycle, regardless of whether the species is diurnally or nocturnally active. Melatonin presumably represents a 'chemical code of the night' for both circadian and seasonal rhythms (Arendt *et al.* 1999).

Immunological role of melatonin

Pineal ablation, or any other experimental procedure that inhibits melatonin synthesis and secretion, such as exposure to constant illumination or pineal denervation, depresses both cellular and humoral immunity that is counteracted partly by exogenous melatonin (Maestroni 2001; Guerrero & Reiter 2002; Skwarlo-Sonta 2002). The immunostimulatory and antiapoptotic role of melatonin is exerted mainly through its action on T-helper lymphocytes (Th). However, it has become increasingly clear that melatonin also acts on T-lymphocyte precursors and affects both natural killer (NK) cell and monocyte function.

The possibility that melatonin could act as an autacoid in the bone marrow is supported by studies which demonstrate that bone marrow cells of both mice and humans are capable of synthesizing melatonin (Conti *et al.* 2000). High concentrations of melatonin are present in bone marrow (Tan *et al.* 1999). However, the formal demonstration of this autacoid role of melatonin in bone marrow, i.e. the inhibition of melatonin activity produces a change within that microenvironment, remains to be documented.

Lymphocytes and melatonin

With respect to lymphocytes specifically, melatonin treatment increases T-lymphocyte proliferation (Pioli *et al.* 1993; Konakchieva *et al.* 1995; Raghavendra *et al.* 2001); enhances antigen presentation by macrophages to T cells by increasing the expression of major histocompatibility complex class-II molecules (Pioli *et al.* 1993); activates splenic, lymph node and bone marrow cells (Wajs *et al.* 1995; Drazen *et al.* 2001); stimulates antibody-dependent cellular cytotoxicity (Giordano & Palermo 1991) and has been shown to augment both innate and adaptive immunity (Poon *et al.* 1994; Bonilla *et al.* 2001; Negrette *et al.* 2001). The most detailed studies have focused on the Th pathway where melatonin increases the number of Th ($CD4^+$) lymphocytes (Lissoni *et al.* 1995; Castrillón *et al.* 2001); restores impaired Th-cell activity in immunosuppressed mice (Maestroni *et al.* 1988); and augments T-cell help as evidenced by antibody responses *in vivo* (Poon *et al.* 1994; Fraschini *et al.* 1998; Akbulut *et al.* 2001).

With respect to cytokine responses, melatonin has been reported to stimulate the production of interleukin (IL)-2, interferon (IFN)-γ and IL-6, but not that of IL-4 (Garcia-Maurino *et al.* 1997; Garcia-Maurino *et al.* 1998; Liu *et al.* 2001) by human peripheral blood mononuclear cells. Melatonin also activates human monocytes to produce IL-1 and IL-12 (Barjavel *et al.* 1998; Lissoni *et al.* 1998; Garcia-Maurino *et al.* 1999). Physiologically, the nocturnal melatonin peak has been associated with high IFN-γ/IL-10 ratio, i.e. the melatonin rhythm correlated with the rhythmicity in the Th1/Th2 ratio (Petrovsky & Harrison 1998). In ischaemic stroke patients, an impaired nocturnal urinary melatonin excretion was found to be associated with a decreased cell-mediated immunity (assessed by skin test); a prevalence of anergic status; and changes in lymphocyte subsets, with an overall decrease in the number of circulating $CD3^+$ lymphocytes (Fiorina *et al.* 1999). Most interesting, reduction of melatonin secretion was reported to parallel disease progression and to correlate with serum IL-12 levels in HIV-1-infected patients (Nunnari *et al.* 2003). These human cytokine studies confirmed that melatonin possesses important immuno-enhancing properties and suggest that melatonin may favour a Th-cell-type 1 response.

The possible connection between IL-2 and melatonin in human lymphocytes has been strengthened recently by the observation that melatonin can be synthesized and released in large quantity by human lymphocytes (Carrillo-Vico *et al.* 2004). Lymphocyte melatonin production seems to be linked strictly to the release of IL-2: inhibition of melatonin synthesis resulted in a decrease of both IL-2 production and IL-2 receptor (IL-2R) expression and addition of exogenous melatonin resulted in an increase of IL-2 production and IL-2R expression. These findings indicate that in addition to the pineal gland, human lymphoid cells are an important physiological source of melatonin and that this melatonin could be involved in the regulation of the human immune system, where it could have an intracrine, autocrine and/or paracrine effect (Garcia-Mauriño *et al.* 1997; Carrillo-Vico *et al.* 2004, 2005). Because IL-2 has been used clinically to enhance T-cell immunity in patients with AIDS or cancer (Nelson 2004), melatonin was also used in an attempt to see whether it would enhance T-cell immunity in cancer patients. The conclusion drawn was that melatonin protects against IL-2 toxicity and synergizes with IL-2 anticancer action. This combined strategy constitutes a novel and well-tolerated form of intervention to control tumour growth. In most patients, both performance status and quality of life were improved (Maestroni 2001).

The ability of melatonin to enhance inflammatory cytokine production (including IL-12) from human monocytes and macrophages (Cutolo *et al.* 1999; Garcia-Maurino *et al.* 1999; Lissoni 2000), together with the association of melatonin with the IL-2-IL-2R mechanisms noted above, suggests that the most relevant role of melatonin in the enhancement

of T-cell immunity is in the control of the Th1 response. Therefore, this Th1-promoting action of melatonin might be exploited in boosting the Th1-priming ability of dendritic cells (DC) loaded with tumour antigens. DCs, the professional antigen-presenting cells, represent attractive vectors for tumour immunotherapy because of their unique properties, which include high antigen capture and presentation capacity, resulting in extremely efficient induction and maintenance of immune responses (Pardoll 1998). Although some promising clinical results have been obtained, current DC-based tumour vaccination methods are cumbersome and not efficacious routinely (Fong & Engleman 2000). Therefore, it would be of interest to explore whether melatonin might help in amplifying the Th1-priming ability of DC-based cancer vaccines.

An alternative effect of melatonin on T cells also needs to be taken into account. In addition to the IL-2 and DC–Th1 pathways, melatonin also prevents apoptosis of T-cell precursors in the thymus (Sainz *et al*. 1995; Provinciali *et al*. 1996). Melatonin appears to act on T-lineage cells throughout all their developmental stages. This antiapoptotic effect may occur via both direct and indirect mechanisms. It may well be that acting via specific receptors, melatonin can directly stimulate Th $CD4^+$ lymphocytes to produce a variety of cytokines and neuropeptides, e.g. κ-opioid cytokine peptides, that are implicated in this (Maestroni & Conti 1996).

The melatonin effects have been explored *in vivo*. The hormone is capable of rescuing haemopoiesis in mice transplanted with Lewis lung carcinoma and treated with cancer chemotherapeutic compounds (Maestroni *et al*. 1994a). This effect apparently involved the endogenous release of granulocyte/macrophage colony-stimulating factor and melatonin-induced opioid cytokines (Maestroni *et al*. 1994a, b, 1999). Both activated lymph node Th cells and bone marrow Th cells release these opioid cytokines (Maestroni *et al*. 1996). This has been confirmed by others in both rats and mice, and using a wide variety of drugs (Anwar *et al*. 1998; Rapozzi *et al*. 1998). In cancer patients, melatonin treatment gave controversial results. Some reports failed to confirm the ability of the hormone to rescue haemopoiesis (Ghielmini *et al*. 1999), but others showed that melatonin not only rescues haemopoiesis but also increases the efficacy of cancer chemotherapy (Lissoni *et al*. 1997; Vijayalaxmi *et al*. 2002; Cerea *et al*. 2003; Lissoni *et al*. 2003).

NK cells and melatonin

As a potential regulator of the immune system, the influence of melatonin on NK cells is of particular interest. NK cells are well-established killers of virus-infected cells and a wide variety of tumour cells, especially those of haemopoietic origin, i.e. leukaemias and lymphomas. It is the spontaneous lytic activity of NK cells against the latter types of neoplasms that have led to the recognition of NK cells several years ago as a possible first line of defense against tumours (Keissling *et al*. 1975; Riccardi *et al*. 1981; Biron & Welsh 1982; Christopher *et al*. 1991), although their role in humans remains controversial. With respect to the mechanism responsible for the influence of melatonin on NK cells, there is evidence in mammals indicating that melatonin, *in vivo*, enhances the lytic function of mature NK cells (Poon *et al*. 1994). However, it remains unknown whether this results directly from receptor–ligand–type interactions between melatonin and NK cell-surface receptors, or indirectly, via melatonin-stimulated, Th-cell-enhanced production of IL-2. Although the mechanism has not been defined directly, one possibility is that melatonin acts through increased IL-2 production, via Th-cell melatonin receptors (Poon *et al*. 1994), and this IL-2 serves as an exquisite stimulant of NK-cell numbers and function (Christopher *et al*. 1991).

In studies in male mice fed every evening (17:00 h) with ground chow, with/without melatonin (24 mg/mouse/day), the haemopoietic cell lineage-specific effects mediated by both short-term (1 week) and longer term (2 weeks) daily melatonin treatment were assessed (Currier *et al*. 2000). The only cell lineages that were augmented quantitatively *in vivo* by melatonin were those that mediated tumour immunosurveillance, i.e. NK cells and monocytes, and monocytes may act in an accessory manner for NK cells via production of NK-stimulating cytokines. Interestingly, the fact that there was a quantitative increment in NK cells in the bone marrow indicates that actual new cell proliferation in this lineage has occurred in the presence of melatonin. All such bone marrow-derived NK cells will traffic unidirectionally via the blood to the spleen, which is their primary destination, and which is where they are found in the greatest number (Miller 1982). Again, direct stimulation of the bone marrow stromal cells, producers of a host of growth-inducing cytokines, may be responsible; or, alternatively, an indirect pathway, via melatonin stimulation of other cells (such as bone marrow-localized monocytes), might be implicated.

In the spleen, as in the bone marrow, NK-cell levels remained elevated significantly after either 1 or 2 weeks of daily melatonin exposure in the diet (Currier *et al*. 2000). Unexpectedly, however, the numbers of monocytes in the spleen did not remain high after 2 weeks of daily, dietary melatonin, despite remaining significantly elevated in the bone marrow at the same period. This suggests that the newly produced monocytes from the bone marrow, capable of acting on NK cells, were located in anatomical sites other than the spleen. These other sites of monocyte-derived

macrophages may also activate NK cells locally, giving rise to an antitumour immune response that could be active in ameliorating a wide variety of neoplasms.

In a subsequent study, erythroleukaemia was induced by intravenous injection of 5×10^6 tumour cells. This leads to death at 16–27 days. Daily dietary administration of melatonin to these mice, beginning at the time of leukaemia onset (e), resulted in a 2.5-fold increase in NK-cell number at 9 days (i.e. at 'mid-stage' leukaemia). All control mice (untreated diet) were dead by 27 days after tumour initiation. Approximately one-third of melatonin-consuming mice remained alive at and beyond 3 months after tumour initiation, indicating long-term survival (Currier & Miller 2001). The results of this study contrast with a report of a negative influence of melatonin on the development of a tumour of haemopoietic origin (a T-cell lymphoma) (Conti *et al.* 1992). However, melatonin is a powerful T-lymphocyte stimulus, promoting the release, of a cascade of T-cell growth factors from the T cells. It seems feasible that the abundance of these factors, resulting from the exogenous administration of melatonin, simply fed the growing T-cell lymphoma. In contrast, in erythroleukaemia, as well as other non-T-cell lymphomas and leukaemias, is not dependent for growth upon factors produced by T cells.

B lymphocytes and melatonin

The role of melatonin in modifying humoral immunity, or cells of the B-lymphocyte lineage that are responsible for mediating humoral immunity, remains undefined (Champney *et al.* 1998; Demas & Nelson 1998). Studies in mice indicated that B lymphocytes are produced throughout life from precursor cells located in the bone marrow (Rolink & Melchers 1993; Osmond *et al.* 1994). This process has built into it a large-scale apoptotic cell death (Lu & Osmond 1997) that constitutes an important quality control mechanism to eliminate aberrant and potentially neoplastic cells, and to regulate the number of new B lymphocytes generated (Melchers *et al.* 1995; Rajewsky 1996). B lymphopoiesis and apoptosis in bone marrow are influenced by a variety of systemic factors (Fauteux & Osmond 1996) and local microenvironmental factors, e.g. the stromal cell-derived cytokine IL-7 (Kincade *et al.* 1994; Valenzona *et al.* 1998).

In a study aimed to assess the possible contribution of melatonin to B-cell homeostasis and B-cell survival in the bone marrow, i.e. specifically the development and selection of precursor B lymphocytes, the effect of melatonin administered in the drinking water was examined *in vivo* (Yu *et al.* 2000). This was measured quantitatively by phenotyping subpopulations of precursor cells in the B lineage and examining their rates of apoptosis using DNA analysis. The results indicated that exogenous melatonin inhibits B-cell apoptosis and that this phenomenon was restricted mainly to the earliest stages of B-cell differentiation. This in turn suggested that melatonin can act as a checkpoint regulator in early B-lymphocyte development and may even contribute to the diurnal rhythm in B-lymphocyte production. Melatonin-induced suppression of apoptosis in the enormous quantities of B cells generated by the bone marrow of the mouse daily could have important pharmacological implications. While it appears that melatonin treatment would result in a greater quantity of B lymphocytes produced (potentially strengthening humoral immunity), such excessive production could permit genetically aberrant B cells to evade the normal deletion process with defective B cells entering the circulation as preneoplastic cells potentially leading, thus, to frank B-cell lymphoma.

Melatonin receptor mRNA is expressed by B lymphocytes in rat spleen and thymus (Pozo *et al.* 1997), and specific binding sites for melatonin have been described in the bursa of Fabricius in birds (Calvo *et al.* 1995). However, melatonin has no direct affinity for B cells, the latter not possessing melatonin receptors, and does not enhance humoral immunity (Champney *et al.* 1998; Demas & Nelson 1998), a phenomenon mediated by mature B lymphocytes. Because melatonin itself is an effective free radical scavenger and antioxidant (Reiter *et al.* 2004), a receptor-independent component of the antiapoptotic effect of the hormone should be considered (Sainz *et al.* 2003).

Melatonin in clinical practice

There are several areas of clinical practice where melatonin may have potential, some of which have been mentioned previously.

As with cells of the immune system, so with melatonin, production is progressively reduced with advancing age. Thus, the potential clinical importance is apparent for maintaining, prophylactically, youthful levels both of melatonin and of cells mediating tumour immunosurveillance, i.e. NK cells. However, liberal use of melatonin in the elderly may need to be revisited. Despite the undoubted value of exogenous melatonin as a sleep regulator and/or inducer, it is nonetheless possible that melatonin in supernormal quantities may produce the undesirable effect of increasing tumour frequency in the elderly. The reason for this is that aberrant cells from the immune system of elderly mammals are produced at higher levels (reflecting factors such as life-long DNA damage) than in younger mammals. Because melatonin inhibits apoptosis, excessive quantities of this hormone could lead to the

development of B-cell lymphomas if enough aberrant B cells escaped the natural deletion process.

For similar reasons, as a pro-inflammatory mediator, melatonin may act to aggravate inflammation and, thus, promote conditions such as rheumatoid arthritis. This would imply a possible contraindication for the use of melatonin in autoimmune diseases (Maestroni *et al*. in press) or, indeed, in any inflammatory condition.

Nonetheless, it appears that for non-T-cell lymphomas and leukaemias and possibly for many yet untested tumours, there is now formal, experimental preclinical evidence for the potential of melatonin in tumour abatement. This evidence is sufficient to justify further studies of the ability of melatonin to favour Th1 priming and/or NK-cell activation, and this might be exploited to boost the efficacy of cancer vaccines in the future.

References

Akbulut K.G., Gonul B., Akbulut H. (2001) The effects of melatonin on humoral immune responses of young and aged rats. *Immunol. Invest.* **30**, 17–20.

Anwar M.M., Mahfouz H.A., Sayed A.S. (1998) Potential protective effects of melatonin on bone marrow of rats exposed to cytotoxic drugs. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **119**, 493–501.

Arendt J., Middleton B., Stone B., Skene D. (1999) Complex effects of melatonin: evidence for photoperiodic responses in humans? *Sleep* **22**, 625–635.

Barjavel M.J., Mamdouh Z., Raghbate N., Bakouche O. (1998) Differential expression of the melatonin receptor in human monocytes. *J. Immunol.* **160**, 1191–1197.

Biron C.A. & Welsh R.M. (1982) Blastogenesis of natural killer cells during viral infection in vivo. *J. Immunol.* **129**, 2788–2795.

Bonilla E., Rodon C., Valero N., Pons H. *et al.* (2001) Melatonin prolongs survival of immunodepressed mice infected with the Venezuelan equine encephalomyelitis virus. *Trans. R. Soc. Trop. Med. Hyg.* **95**, 207–210.

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

Carrillo-Vico A., Calvo J.R., Abreu P. *et al.* (2004) Evidence of melatonin synthesis by human lymphocytes and its physiological significance: possible role as intracrine, autocrine, and/or paracrine substance. *FASEB J.* **18**, 537–539.

Carrillo-Vico A., Lardone P.J., Fernandez-Santos J.M. *et al.* (2005) Human lymphocyte-synthesized melatonin is involved in the regulation of the IL-2/IL-2 receptor system. *J. Clin. Endocrinol. Metab.* **90**, 992–1000.

Castrillón P., Cardinali D.P., Pazo D., Cutrera R.A., Esquifino A.I. (2001) Effect of superior cervical ganglionectomy on 24-h variations in hormone secretion from anterior hypophysis and in hypothalamic monoamine turnover, during the preclinical phase of Freund's adjuvant arthritis in rats. *J. Neuroendocrinol.* **13**, 288–295.

Cerea G., Vaghi M., Ardizzoia A. *et al.* (2003) Biomodulation of cancer chemotherapy for metastatic colorectal cancer: a randomized study of weekly low-dose irinotecan alone versus irinotecan plus the oncostatic pineal hormone melatonin in metastatic colorectal cancer patients progressing on 5-fluorouracil-containing combinations. *Anticancer Res.* **23**, 1951–1954.

Champney T.H., Allen G.C., Zannelli M., Beausang L.A. (1998) Time-dependent effects of melatonin on immune measurements in male Syrian hamsters. *J. Pineal. Res.* **25**, 142–146.

Christopher F.L., Dussault I., Miller S.C. (1991) Population dynamics of natural killer cells in the spleen and bone marrow of normal and leukemic mice during in vivo exposure to interleukin-2. *Immunobiology* **184**, 37–52.

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

Conti A., Haran-Ghera N., Maestroni G.J. (1992) Role of pineal melatonin and melatonin-induced-immuno-opioids in murine leukemogenesis. *Med. Oncol. Tumor Pharmacother.* **9**, 87–92.

Currier N.L. & Miller S.C., (2001) Echinacea purpurea and melatonin augment natural-killer cells in leukemic mice and prolong life span. *J. Altern. Complement Med.* **7**, 241–251.

Currier N.L., Sun L.Z., Miller S.C. (2000) Exogenous melatonin: quantitative enhancement in vivo of cells mediating nonspecific immunity. *J. Neuroimmunol.* **104**, 101–108.

Cutolo M., Villaggio B., Candido F. *et al.* (1999) Melatonin influences interleukin-12 and nitric oxide production by primary cultures of rheumatoid synovial macrophages and THP-1 cells. *Ann. N. Y. Acad. Sci.* **876**, 246–254.

Demas G.E. & Nelson R.J. (1998) Exogenous melatonin enhances cell-mediated, but not humoral, immune function in adult male deer mice (Peromyscus maniculatus). *J. Biol. Rhythms* **13**, 245–252.

Drazen D.L., Bilu D., Bilbo S.D., Nelson R.J. (2001) Melatonin enhancement of splenocyte proliferation is attenuated by luzindole, a melatonin receptor antagonist. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **280**, R1476–R1482.

Fauteux L.J. & Osmond D.G. (1996) IL-1 as systemic modifier of B lymphopoiesis. Recombinant IL-1 alpha binds to stromal cells and sinusoid endothelium in bone marrow and precursor B cell dynamics. *J. Immunol.* **156**, 2376–2383.

Fiorina P., Lattuada G., Silvestrini C., Ponari O., Dall'Aglio P. (1999) Disruption of nocturnal melatonin rhythm and immunological involvement in ischaemic stroke patients. *Scand. J. Immunol.* **50**, 228–231.

Fong L. & Engleman E.G. (2000) Dendritic cell in cancer immunotherapy. *Annu. Rev. Immunol.* **18**, 245–273.

Fraschini F., Demartini G., Esposti D., Scaglione F. (1998) Melatonin involvement in immunity and cancer. *Biol. Signals* **7**, 61–72.

Garcia-Maurino S., Gonzales-Haba M.G., Calvo J.R., Goberna R., Guerrero J.-M. (1998) Involvement of nuclear binding sites for melatonin in the regulation of IL-2 and IL-6 production in human blood mononuclear cells. *J. Neuroimmunol.* **92**, 76–84.

Garcia-Mauriño S., Gonzales-Haba M.G., Calvo J.R. *et al.* (1997) Melatonin enhances IL-2, IL-6, and IFN-γ production by human circulating CD4+ cells. *J. Immunol.* **159**, 574–581.

Garcia-Maurino S., Gonzalez-Haba M.G., Calvo J.R. *et al.* (1997) 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 1 lymphocytes and monocytes. *J. Immunol.* **159**, 574–581.

Garcia-Maurino S., Pozo D., Carrillo-Vico A., Calvo J.R., Guerrero J.M. (1999) Melatonin activates Th1 lymphocytes by increasing IL-12 production. *Life Sci.* **65**, 2143–2150.

Ghielmini M., Pagani O., de Jong J. *et al.* (1999) Double-blind randomized study on the myeloprotective effect of melatonin in combination with carboplatin and etoposide in advanced lung cancer. *Br. J. Cancer* **80**, 1058–1061.

Giordano M. & Palermo M.S. (1991) Melatonin-induced enhancement of antibody-dependent cellular cytotoxicity. *J. Pineal Res.* **10**, 117–121.

Guerrero J.M. & Reiter R.J. (2002) Melatonin-immune system relationships. *Curr. Top Med. Chem.* **2**, 167–179.

Keissling R., Klein E., Pross H., Wigzell H. (1975) Natural killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells: characteristics of the killer cell. *Eur. J. Immunol.* **5**, 117–121.

Kincade P.W., Medina K.L., Smithson G. (1994) Sex hormones as negative regulators of lymphopoiesis. *Immunol. Rev.* **137**, 119–134.

Konakchieva R., Kyurkchiev S., Kehayov I., Taushanova P., Kanchev L. (1995) Selective effect of methoxyindoles on the lymphocyte proliferation and melatonin binding to activated human lymphoid cells. *J. Neuroimmunol.* **63**, 125–132.

Lissoni P. (2000) Modulation of anticancer cytokines IL-2 and IL-12 by melatonin and the other pineal indoles 5-methoxytryptamine and 5-methoxytryptophol in the treatment of human neoplasms. *Ann. N. Y. Acad. Sci.* **917**, 560–567.

Lissoni P., Barni S., Brivio F. *et al.* (1995) A biological study on the efficacy of low-dose subcutaneous interleukin-2 plus melatonin in the treatment of cancer-related thrombocytopenia. *Oncology* **52**, 360–362.

Lissoni P., Chilelli M., Villa S., Cerizza L., Tancini G. (2003) Five years survival in metastatic non-small cell lung cancer patients treated with chemotherapy alone or chemotherapy and melatonin: a randomized trial. *J. Pineal. Res.* **35**, 12–15.

Lissoni P., Paolorossi F., Ardizzoia A. *et al.* (1997) A randomized study of chemotherapy with cisplatin plus etoposide versus chemoendocrine therapy with cisplatin, etoposide and the pineal hormone melatonin as a first-line treatment of advanced non-small cell lung cancer patients in a poor clinical state. *J. Pineal. Res.* **23**, 15–19.

Lissoni P., Rovelli F., Brivio F., Brivio O., Fumagalli L. (1998) Circadian secretions of IL-2, IL-12, IL-6 and IL-10 in relation to the light/dark rhythm of the pineal hormone melatonin in healthy humans. *Nat. Immun.* **16**, 1–5.

Liu F., Ng T.B., Fung M.C. (2001) Pineal indoles stimulate the gene expression of immunomodulating cytokines. *J. Neural Transm.* **108**, 397–405.

Lu L. & Osmond D.G. (1997) Apoptosis during B lymphopoiesis in mouse bone marrow. *J. Immunol.* **158**, 5136–5145.

Maestroni G.J. (2001) The immunotherapeutic potential of melatonin. *Expert Opin. Investig. Drugs* **10**, 467–476.

Maestroni G.J.M., Cardinali D.P., Esquifino A.I., Pandi-Perumal S.R. Does melatonin play a disease-promoting role in rheumatoid arthritis? J. Neuroimmunol. **158**, 106–111.

Maestroni G.J. & Conti A. (1996) Melatonin and the immune-hematopoietic system therapeutic and adverse pharmacological correlates. *Neuroimmunomodulation* **3**, 325–332.

Maestroni G.J., Conti A., Lissoni P. (1994a) 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., Covacci V., Conti A. (1994b) Hematopoietic rescue via T-cell-dependent, endogenous granulocyte- macrophage colony-stimulating factor induced by the pineal neurohormone melatonin in tumor-bearing mice. *Cancer Res.* **54**, 2429–2432.

Maestroni G.J., Conti A., Pierpaoli W. (1988) Pineal melatonin, its fundamental immunoregulatory role in aging and cancer. *Ann. N Y Acad. Sci.* **521**, 140–148.

Maestroni G.J., Hertens E., Galli P., Conti A., Pedrinis E. (1996) Melatonin-induced T-helper cell hematopoietic cytokines resembling both interleukin-4 and dynorphin. *J. Pineal. Res.* **21**, 131–139.

Maestroni G.J., Zammaretti F., Pedrinis E. (1999) Hematopoietic effect of melatonin involvement of type 1 kappa-opioid receptor on bone marrow macrophages and interleukin-1. *J. Pineal. Res.* **27**, 145–153.

Melchers F., Rolink A., Grawunder U. *et al.* (1995) Positive and negative selection events during B lymphopoiesis. *Curr. Opin. Immunol.* **7**, 214–227.

Miller S.C. (1982) Production and renewal of murine natural killer cells in the spleen and bone marrow. *J. Immunol.* **129**, 2282–2286.

Negrette B., Bonilla E., Valero N. *et al.* (2001) Melatonin treatment enhances the efficiency of mice immunization with Venezuelan equine encephalomyelitis virus TC-83. *Neurochem. Res.* **26**, 767–770.

Nelson B.H. (2004) IL-2, regulatory T cells, and tolerance. *J. Immunol.* **172**, 3983–3988.

Nunnari G., Nigro L., Palermo F., Leto D., Pomerantz R.J., Cacopardo B. (2003) Reduction of serum melatonin levels in HIV-1-infected individuals' parallel disease progression: correlation with serum interleukin-12 levels. *Infection* **31**, 379–382.

Osmond D.G., Rico-Vargas S., Valenzona H. *et al.* (1994) Apoptosis and macrophage-mediated cell deletion in the regulation of B lymphopoiesis in mouse bone marrow. *Immunol. Rev.* **142**, 209–230.

Pardoll D.M. (1998) Cancer vaccines. *Nat. Med.* **4**, 525–531.

Petrovsky N. & Harrison L. (1998) The chronobiology of human cytokine production. *Int. Rev. Immunol.* **16**, 635–649.

Pioli C., Caroleo M.C., Nistico 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.

Poon A.M., Liu Z.M., Pang C.S., Brown G.M., Pang S.F. (1994) Evidence for a direct action of melatonin on the immune system. *Biol. Signals* **3**, 107–117.

Pozo D., Delgado M., Fernandez-Santos J.M. *et al.* (1997) Expression of the Mel1a-melatonin receptor mRNA in T and B subsets of lymphocytes from rat thymus and spleen. *FASEB J.* **11**, 466–473.

Provinciali M., Di Stefano G., Bulian D., Tibaldi A., Fabris N. (1996) Effect of melatonin and pineal grafting on thymocyte apoptosis in aging mice. *Mech. Ageing Dev.* **90**, 1–19.

Raghavendra V., Singh V., Shaji A.V., Vohra H., Kulkarni S.K., Agrewala J.N. (2001) Melatonin provides signal 3 to unprimed CD4+ T cells but failed to stimulate LPS primed B cells. *Clin. Exp. Immunol.* **124**, 414–422.

Rajewsky K. (1996) Clonal selection and learning in the antibody system. *Nature* **381**, 751–758.

Rapozzi V., Zorzet S., Comelli M., Mavelli I., Perissin L., Giraldi T. (1998) Melatonin decreases bone marrow and lymphatic toxicity of adriamycin in mice bearing TLX5 lymphoma. *Life Sci.* **63**, 1701–1713.

Reiter R.J., Tan D.X., Gitto E. *et al.* (2004) Pharmacological utility of melatonin in reducing oxidative cellular and molecular damage. *Pol. J. Pharmacol.* **56**, 159–170.

Riccardi C., Barlozzari T., Santoni A., Herberman R.B., Cesarini C. (1981) Transfer to cyclophosphamide-treated mice of natural killer (NK) cells and in vivo natural reactivity against tumors. *J. Immunol.* **126**, 1284–1289.

Rolink A. & Melchers F. (1993) B lymphopoiesis in the mouse. *Adv. Immunol.* **53**, 123–156.

Sainz R.M., Mayo J.C., Reiter R.J., Tan D.X., Rodriguez C. (2003) Apoptosis in primary lymphoid organs with aging. *Microsc. Res. Tech* **62**, 524–539.

Sainz R.M., Mayo J.C., Uria H. *et al.* (1995) The pineal neurohormone melatonin prevents in vivo and in vitro apoptosis in thymocytes. *J. Pineal. Res.* **19**, 178–188.

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

Tan D.X., Manchester L.C., Reiter R.J. *et al.* (1999) Identification of highly elevated levels of melatonin in bone marrow: its origin and significance. *Biochim. Biophys. Acta* **1472**, 206–214.

Valenzona H.O., Dhanoa S., Finkelman F.D., Osmond D.G. (1998) Exogenous interleukin 7 as a proliferative stimulant of early precursor B cells in mouse bone marrow: efficacy of IL-7 injection, IL-7 infusion and IL-7-anti-IL-7 antibody complexes. *Cytokine* **10**, 404–412.

Thomas C.R.V. Jr, Reiter R.J., Herman T.S. (2002) Melatonin: from basic research to cancer treatment clinics. *J. Clin. Oncol.* **20**, 2575–2601.

Wajs E., Kutoh E., Gupta D. (1995) Melatonin affects proopiomelanocortin gene expression in the immune organs of the rat. *Eur. J. Endocrinol.* **133**, 754–760.

Yu Q., Miller S.C., Osmond D.G. (2000) Melatonin inhibits apoptosis during early B-cell development in mouse bone marrow. *J. Pineal. Res.* **29**, 86–93.