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Inhibitory effect of melatonin on production of IFNγ or TNFα in peripheral blood mononuclear cells of some blood donors

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Abstract: Melatonin, the main pineal hormone, has been shown to influence many biological functions, including the immune response and cancer growth. The purpose of this study was to examine the effect of melatonin on the production of interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα) by peripheral blood mononuclear cells (PBMC) in culture. Melatonin at physiological concentrations fails to induce production of IFN or TNF by PBMC in culture but causes a dose-related inhibition of production of both cytokines if the PBMC are stimulated with phytohaemagglutinin. This inhibitory effect occurs in only 22% of cases (melatonin-sensitive) but disappears when the cells are stored at 4°C for 4 days. The effect of melatonin appears not to be mediated by opiates nor to be correlated with the age, sex, or blood group of donors, but seems to be influenced by the seasonal time of blood collection. These results provide further evidence for an interaction between melatonin and the immune system and suggest that the effect of melatonin on production of IFN and TNF may be mediated by various factors not yet fully understood.

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Introduction

Melatonin, the main pineal hormone, has been shown to influence many biological systems, including the immune system. Melatonin has many pleiotropic activities: it is an important conveyor of photoperiodic information to the organism [Bartness and Goldman, 1989], influences hematopoiesis [Vaughan et al. 1987], modifies life span in aging mice [Pierpaoli and Yi, 1990], is able to modulate the immune response [Maestroni et al., 1986; Becker et al., 1988; Del Gobbo et al., 1989], inhibits tumor growth [Lissoni et al., 1993a; Maestroni et al., 1988a] and may improve the quality of life [Maestroni et al., 1989]. Daily administration of melatonin counteracts the effect of acute stress and may induce tumor regression [Lissoni et al., 1989]. It has been reported that psychiatric diseases and affective disorders also may be associated with altered melatonin production [Brambilla et al., 1988].

There is evidence of a relationship between the positive effect of melatonin on control of tumor growth and its immunomodulatory effect [Lissoni et al., 1993b]. It has been reported that melatonin influences both humoral and cell-mediated immunological processes. In fact, interleukin 2 (IL-2) production [Libri et al., 1990] and antigen presentation [Pioli et al., 1993] are stimulated by melatonin, whereas the release of interferon gamma (IFNγ) has been reported both to be stimulated [Colombo et al., 1992] and to be depressed [Artz et al., 1988] by melatonin. The immunological effect of melatonin seems to be mediated by an opiatergic mechanism [Maestroni et al., 1988b; Garza et al., 1993].

To gain more understanding of the mechanism of action of melatonin on the immune response, we investigated the possible modulation by melatonin of the release of tumor necrosis factor (TNF) and IFN by fresh and "stored" peripheral blood mononuclear cells (PBMC) from healthy human subjects.

Materials and methods

PBMC preparation

Peripheral venous blood (100 to 200 ml) was withdrawn from 23 healthy donors, ranging in age from 25 to 60 years, who were negative for hepatitis B surface antigen and HIV. Blood samples were diluted 1:1 with phosphate buffered saline (PBS) supplemented with heparin (10 IU/ml) and layered over Ficoll-Hypaque (Seromed, Biochrom KG, Germany) for separation of PBMC according to the method of Boyum (1968). The PBMC fraction was resuspended in RPMI 1640 medium (DUTCH modified, Flow Laboratories, Irvine, Scotland), supplemented with 10% heat-inactivated (56°C, 60 min) fetal calf serum (FCS) (Seromed), penicillin (100 U/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM), at the final concentration of 1.5×10^6 viable cells/ml. Cell viability was determined by the trypan blue exclusion technique and light microscope observation.

Treatment of samples

PBMC suspensions were plated in 48-well culture plates (Costar, Badhoevedorp, The Netherland), 0.5 ml per well. Some of the plates were sealed with an adhesive plate cover, stored for 4 days at 4°C, and then processed in the same way as the fresh cells.

The melatonin preparation (Fluka, Chemika-Biochemica, Buchs, Switzerland) was dissolved in endotoxin-free sterile water in a warm bath and then diluted in RPMI 1640, containing penicillin, streptomycin, glutamine, and 10% FCS, and added to the cell suspensions at final molar concentrations of 10^{-6}, 10^{-9}, 10^{-12} and 10^{-15}. The PBMC suspensions treated with melatonin were incubated at 37°C in a humidified atmosphere (95% air 5% CO_2) for 12 hr, after which phytohaemagglutin (PHA) was added to them and the cell suspensions were further incubated. At 24 and 48 hr after the addition of PHA, samples were centrifuged at $1{,}000 \times g$ for 10 min at 4°C and the supernatants were frozen at −80°C until IFN and TNF determination.

Finally naloxone (Sigma Chemical Co., St. Louis, MO), which is known to inhibit the release of opioids induced by melatonin, was added to the cell suspensions at the final concentration of 5×10^{-5} M. Controls underwent the same treatment except that PBS was added instead of melatonin. All the procedures were carried out in a darkened room to avoid melatonin alteration by light.

IFN determination and characterization

Supernatants were titrated for IFN activity with an antiviral assay [Langford et al., 1981] using human amniotic cells (Wish) and vesicular stomatitis virus (VSV, Indiana strain) as the challenge virus. Titrations were always performed using the international reference preparations for human IFNα (Ga23-902-530), human IFNβ (G023-902-527), and human IFNγ (Gg23-901-530) (NIAID, Bethesda, MD). Titers were expressed as International Units (IU) per ml. Neutralization of IFN was carried out as follows: samples of IFN (100 IU/ml) and of the international standards (100 IU/ml) were incubated for 60 min at 37°C in the presence of individual and pooled anti-IFNα, anti-IFNβ, and anti-IFNγ antisera (NIAID) and the residual antiviral activity was then assayed. For each experiment, the amounts of the antisera used were sufficient to neutralize completely the corresponding IFNs.

TNFα determination

Concentrations of TNFα were measured simultaneously by a sandwich enzyme immunoassay (BIOKINE TNF test kit, T Cell Sciences, Cambridge, MA) and by a bioassay using L929 and actinomycin D [Ruff and Gifford, 1981]. Both tests are highly specific for TNFα activity. Having calculated a conversion factor by using a TNFα standard solution measured by bioassay, we could report the biological activity (expressed in U/ml), as pg/ml.

ELISA assay for TNF-α

A sandwich enzyme immunoassay was used to determine TNFα Ag concentration in PBMC culture supernatants. Briefly, samples were incubated for 2 hr at 37°C in a air incubator in microtiter wells previously absorbed with a mouse anti-human TNF monoclonal antibody.

After washing, an enzyme conjugated monoclonal antibody with neutralizing properties against TNF was added to the samples and they were incubated for an additional 2 hr. A washing step removed the unbound enzyme conjugated anti-TNF monoclonal antibody and a substrate solution was added to the wells. The colored product, formed in proportion to the amount of TNF present in the samples, was read at 490 nm versus a substrate blank on a Titertek Multiskan microElisa reader (Flow Laboratories). The cytokine concentrations in samples were expressed in pg/ml determined with a standard curve generated from samples of known TNF concentrations.

TABLE 1. Percentage of the IFNγ production by PHA-induced PBMC suspensions treated with different concentrations of MLT[a]

	IFNγ (% of production)[b]			
	MLT [10^{-6}]	MLT [10^{-9}]	MLT [10^{-12}]	MLT [10^{-15}]
MLT-sensitive PBMC (n = 7)	6.39 ± 1.68	24.30 ± 8.60	58.40 ± 8.79	86.28 ± 6.01
non-MLT-sensitive PBMC (n = 16)	95.30 ± 4.30	97.68 ± 4.19	96.62 ± 5.44	97.10 ± 2.99

[a]PBMC suspensions treated with melatonin (MLT) were incubated for 12 hr at 37°C in air/CO_2, then PHA (5 μg/ml) was added to them and they were incubated for a further 24 hr. Supernatants were titrated for IFN by an antiviral assay.
[b]Percentage of IFN production was determined by considering the value for the cultures without MLT (controls) as 100%. Values are reported as mean ± SD.

TABLE 2. Percentage of the TNFα production by PHA-induced PBMC suspensions treated with different concentrations of MLT[a]

	TNFα (% of production)[b]			
	MLT [10^{-6}]	MLT [10^{-9}]	MLT [10^{-12}]	MLT [10^{-15}]
MLT-sensitive PBMC (n = 7)	9.24 ± 5.58	19.13 ± 5.02	47.66 ± 17.80	72.81 ± 11.25
non-MLT-sensitive PBMC (n = 16)	98.00 ± 3.59	97.25 ± 4.60	98.06 ± 3.77	96.43 ± 4.15

[a]PBMC suspensions treated with melatonin (MLT) were incubated for 12 hr at 37°C in air/CO_2, then PHA (5 μg/ml) was added to them and they were incubated for a further 24 hr. Supernatants were titrated for TNF by an immunoassay and a biological assay.
[b]Percentage of TNF production was determined by considering the value for the cultures without MLT (controls) as 100%. Values are reported as mean ± SD.

The limit of sensitivity of this assay was 10 pg/ml.

Biological assay

The biological activity was determined by the method of Ruff and Gifford [1981] with some modifications. Briefly, L929 cells were seeded at a density of 4×10^4 cells per well in 96-well culture plates in 100 μl of EMEM supplemented with 10% FCS and antibiotics. After 4 hr of incubation at 37°C in a humidified atmosphere (5%CO_2–95% air), twofold serial dilutions of the samples in actinomycin D (Serva, Heidelberg) 2 μg/ml were prepared in separate 96-well culture dishes. Then, 100 μl samples of each dilution were transferred into the corresponding wells and plates and were further incubated for 18 hr at 37°C in the humidified CO_2 incubator.

Supernatants were then removed and cells were stained with 0.1% crystal violet. After drying, 100 μl of 33% acetic acid was added to each well to dissolve the dye. Plates were finally read at 540 nm on a Titertek Multiskan microElisa reader. Units of TNF activity were defined as the reciprocal of the dilution causing 50% of maximum cytotoxicity.

Results

The first series of experiments was designed to determine if melatonin is able to induce IFN and TNF production from PBMC suspensions. Since we did not detect any IFN or TNF, melatonin was then tested for its ability to influence the production of both cytokines by PBMC stimulated with PHA; suspensions of PBMC with concentrations of melatonin ranging from 10^{-6} to 10^{-15} were challenged with 5 μg/ml PHA 12 hr after the addition of MLT, and the supernatants were tested for the presence of IFN and TNF.

In our experiments the response to melatonin was highly variable (Tables 1 and 2). In fact the PBMC of only 22% of the subjects (melatonin-sensitive) exhibited significant inhibition of the release of both IFN and TNF (two tailed t-test), while the PBMC from the other 78% (non-melatonin-sensitive) did not show any significant difference from the controls. The characteristics of the donors and the time of blood collection reported in Table 3 indicate that the melatonin responsiveness may be influenced by the time of year that blood was collected, whereas the age, sex, and the blood group of the donors do not seem to have any effect. In the melatonin-sensitive PBMC (Figs. 1 and 2) the degree of inhibition was dose-dependent at both 24 and 48 hr of incubation.

To investigate a possible relationship between melatonin and aging in vitro, some PBMC suspensions were sealed and stored at 4°C for 4 days. This in vitro storage did not significantly modify the cell responsiveness to PHA (Fig. 3). However, in these conditions, no inhibitory effect of melatonin was observed on the release of either IFN (Fig. 4) or TNF (Fig. 5) even in the PBMC of melatonin-sensitive donors.

TABLE 3. Characteristics of PBMC donors

	MLT-sensitive	non-MLT-sensitive
Age-mean (range)	45 (25–60)	37 (27–53)
Sex (no. of donors)	men (4) women (3)	men (10) women (6)
Blood group (no. of donors)	0, Rh^+ (4); A, Rh^+ (3)	0, Rh^+(7); 0, Rh^- (2); A, Rh^+(4); A, Rh^- (2); AB, Rh^+(1)
Month of blood collection (no. of donors)	Jan. (3); Feb. (2); Mar. (2)	May (3); Jun. (3); Oct. (3); Nov. (4); Dec. (1); Mar. (2)
Daily time of blood collection[a]	8–10 AM	8–10 AM

[a]PBMC suspensions were quickly isolated soon after blood collection and treated with melatonin (MLT) at 6–8 PM of the same day.

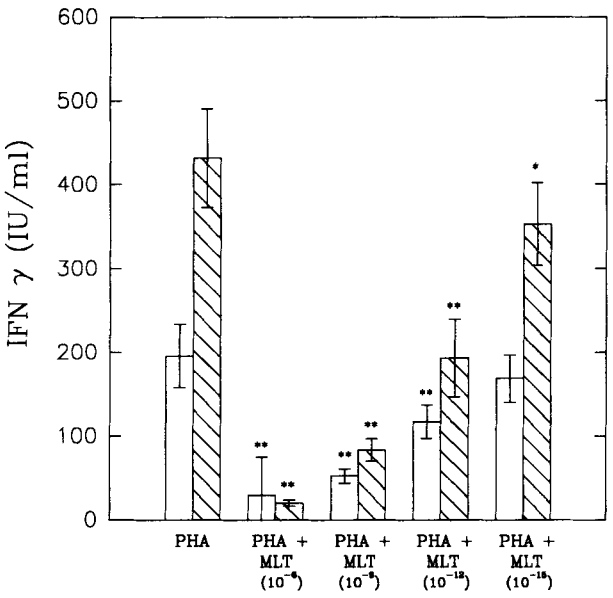

Fig. 1. Effect of different concentrations of melatonin on the release of IFNγ by PHA-induced melatonin-sensitive PBMC. Cells were incubated for 24 (□) or 48 (□) hr after the addition of PHA. Values are reported as mean ± SD. $*P < 0.05$; $**P < 0.005$.

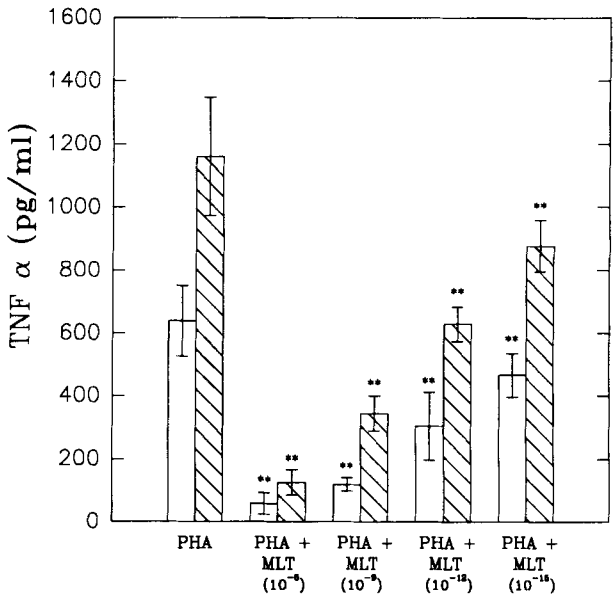

Fig. 2. Effect of different concentrations of melatonin on the release of TNFα by PHA-induced melatonin-sensitive PBMC. Cells were incubated for 24 (□) or 48 (□) hr after the addition of PHA. Values are reported as mean ± SD. $*P < 0.05$; $**P < 0.005$.

The possible role of opiates in mediating the inhibitory mechanism of action of melatonin on cytokine production was investigated by studying the effect of naloxone on the release of IFN and TNF from melatonin-sensitive PBMC. In our experiments naloxone did not counteract the inhibitory effect of melatonin seen in the responsive cells (data not shown), indicating that opioid-agonist substances are not involved in this process.

Discussion

A growing body of evidence suggests that melatonin modulates the immune response, inhibits tumor growth, and modifies life span in aged mice [Bartsch et al., 1992; Lissoni et al., 1993a; Pierpaoli and Yi, 1990]. However, despite the great number of studies concerning the relationship between melatonin and the immune response, very little, and contradictory, information is available concerning the effect of melatonin on human lymphocytes in vitro.

In this report we have shown that melatonin, at physiological concentrations ranging from 10^{-6} to 10^{-15} M, is completely ineffective in inducing the production of IFN or TNF by lymphomonocytes in culture. The failure of melatonin to induce cytokine production in vitro is consistent with other studies, which document that melatonin is effective only in cases in which the immune system is activated by appropriate stimuli or when cells are from immunodepressed subjects [Lissoni et al., 1993b]. In fact in our experiments, melatonin inhibited IFN and TNF production by PHA-induced PBMC suspensions in a dose-dependent response, even though this effect occurred only in 22% of cases. This

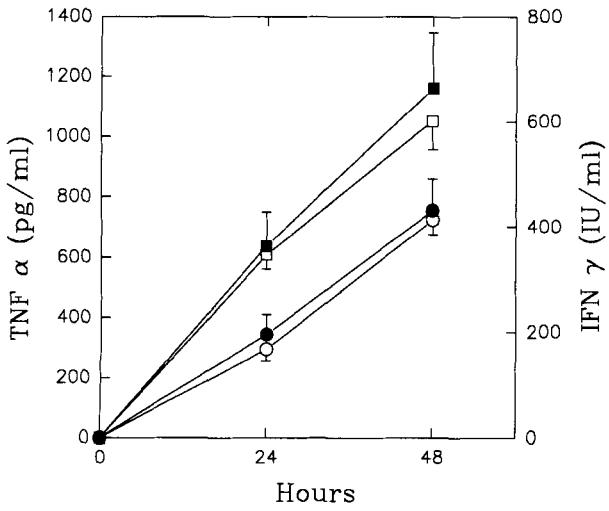

Fig. 3. Production of IFNγ(○ ●) and TNFα (□ ■) by fresh (empty symbols) and "stored" (full symbols) PHA-induced PBMC.

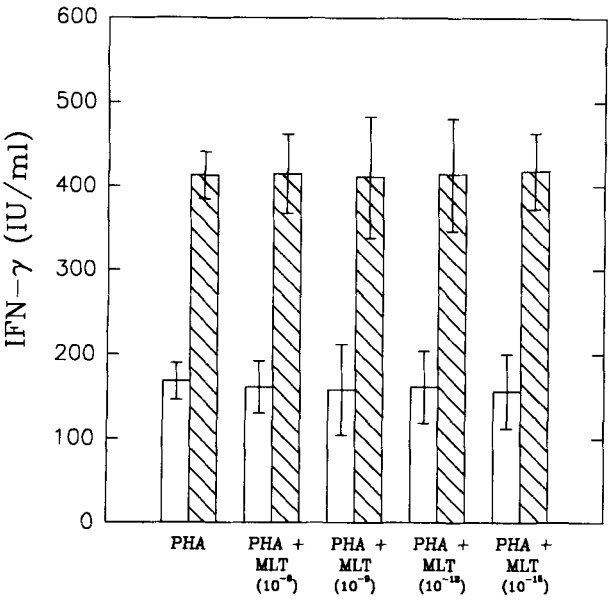

Fig. 4. Production of IFNγ by PHA-induced melatonin-sensitive PBMC, kept for 4 days at 4°C and treated with melatonin at different molar concentrations. Cells were incubated for 24 (□) or 48 (□) hr after the addition of PHA. Values are reported as mean ± SD.

variability is in agreement with Maestroni and Conti [1990], who reported that only 4 out of 10 blood donors respond to melatonin. Moreover, the inhibitory effect of melatonin found by us is in agreement with recent reports showing that melatonin inhibits IFNγ production by PBMC [Artz et al., 1988] and induces immunosuppression of human lymphocyte NK activity in vitro [Lewinski et al., 1989]. In contrast, other reports have demonstrated that me-

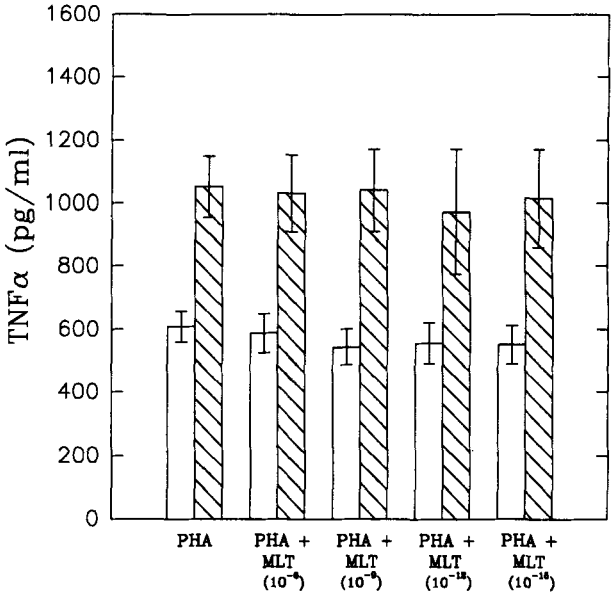

Fig. 5. Production of TNFα by PHA-induced melatonin-sensitive PBMC, kept for 4 days at 4°C and treated with melatonin at different molar concentrations. Cells were incubated for 24 (□) or 48 (□) hr after the addition of PHA. Values are reported as mean ± SD.

latonin increases the expression of MHC class II antigens [Colombo et al., 1992] and the production of cytokines [Pioli et al., 1993].

Thus, the picture that emerges from the study of the interaction between melatonin and the immune system in vitro is that melatonin modulates the immune response only under certain conditions that are not yet fully understood.

The difference in response among different donors could be a result of unknown factors, such as the subject's hormonal and/or immunological status [Lissoni et al., 1993b] and the melatonin receptor expression on the cell membrane [Morgan and Williams, 1989], or by known factors, such as the age of donors and the daily or seasonal time of blood collection [Colombo et al., 1992; Ritchie et al., 1983]. Our experiments seem to indicate that seasonal variations may influence the inhibitory effect of melatonin on the production of cytokines.

Contrary to other reports [Maestroni et al., 1988b; Garza et al., 1993], we have shown that naloxone does not counteract the inhibitory effect of melatonin, suggesting that melatonin acts either directly on the production of IFN and TNF without the involvement of opioid agonists or by modulation of cytokines, which have an inhibitory action on IFN and TNF release [Chantry et al., 1989; Fiorentino et al., 1991]. Finally labile factors might also be involved in the action of melatonin, since after melatonin-sensitive PBMC were kept for 4

days at 4°C, there was no longer any effect of melatonin on the release of the two cytokines.

In conclusion, the present report provides further evidence of a complex interaction between melatonin and the immune system in vitro. However various factors probably influence the effect of melatonin on the release of cytokines from PBMC.

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Literature cited

ARTZ, E.S., S. FERNANDEZ-CASTELO, L.M.E. FINOCCHIARO, M.E. CRISCUOLO, A. DIAZ, S. FINKIELMAN, V.E. NAHMOD (1988) Immunomodulation by indoleamines: serotonin and melatonin action on DNA and interferon-γ synthesis by human peripheral blood mononuclear cells. J. Clin. Immunol. 8:513–520.

BARTSCH, C., H. BARTSCH, T.H. LIPPERT (1992) The pineal gland and cancer: facts, hypotheses and perspectives. Cancer J. 5:194–199.

BARTNESS, T.J., B.D. GOLDMAN (1989) Mammalian pineal melatonin: a clock for all seasons. Experientia 45:939–945.

BECKER, J., G. VEIT, R. HANDGRETINGER, A. ATTANASIO, G. BRUCHETT, I. TRENNER, D. NIETHAMMER, D. GUPTA (1988) Circadian variations in the immunomodulatory role of the pineal gland. Neuroendocrinol. Lett. 10:65–80.

BOYUM, W. (1968) Separation of lymphocytes from peripheral blood and bone marrow. Scand. J. Clin. Invest. 21 (Suppl. 97):77–81.

BRAMBILLA, F., F. FRASCHINI, G. ESPOSTI, P.A. BOSSOLO, G. MARELLI, E. FERRARI (1988) Melatonin circadian rhythm in anorexia nervosa and obesity. Psychiat. Res. 23:267–274.

CHANTRY, D., M. TURNER, E. ABNEY, M. FELDMAN (1989) Modulation of cytokine production by transforming growth factor-β^1. J. Immunol. 142:4295–4300.

COLOMBO, L.L., G.-J. CHEM, M.C. LOPEZ, R.R. WATSON (1992) Melatonin induced increase in gamma-interferon production by murine splenocytes. Immunol. Lett. 33:123–126.

DEL GOBBO, V., V. LIBRI, N. VILLANI, R. CALIO, G. NISTICO (1989) Pinealectomy inhibits interleukin-2 production and natural killer activity in mice. Int. J. Immunopharmacol. 11:567–577.

FIORENTINO, D.F., A. ZLOTNIK, T.R. MOSMANN, M. HOWARD, A. O'GARRA (1991) IL-10 Inhibits cytokine production by activated macrophages. J. Immunol. 147:3815–3822.

GARZA, H.H. JR., B. STANKOV, F. FRASCHINI, D.J.J. CARR (1993) The relationship between opioids and melatonin in the immune system. Neuroendocrinol. Lett. 15:135–148.

LANGFORD, M.P., D.A. WEIGENT, G.J. STANTON, S. BARON (1981) Virus plaque reduction assay for interferon: microplaque and regular macroplaque reduction assay. In: Methods in Enzymology 78A. S. Pestka ed. Academic Press, New York, pp. 339–346.

LEWINSKI, A., P. ZELAZOWSKY, E. SEWERYNEK, G. ZEREK-MELEN, M. SZKUDLINSKI (1989) Melatonin-induced immunosuppression of human lymphocyte natural killer activity in vitro. J. Pineal Res. 7:153–163.

LIBRI, V., V. DEL GOBBO, N. VILLANI, R. CALIO, G. NISTICO (1990) Influence of pineal gland lesion on interleukin-2 production and natural killer activity in C57BL/6 mice. Pharmacol. Res. 22:52.

LISSONI, P., S. BARNI, S. CRISPINO, G. TANCINI, F. FRASCHINI (1989) Endocrine and immune effects of melatonin therapy in metastatic cancer patients. Eur. J. Cancer Clin. Oncol. 25:789–795.

LISSONI, P., S. BARNI, G. TANCINI, A. ARDIZZOIA, F. ROVELLI, M. CAZZANIGA, F. BRIVIO, A. PIPERNO, R. ALDEGHI, D. FOSSATI, D. CHARACIEJUS, L. KOTHARI, A. CONTI, G.J.M. MAESTRONI (1993a) Immunotherapy with subcutaneous low-dose interleukin-2 and the pineal indole melatonin as a new effective therapy in advanced cancers of the digestive tract. Br. J. Cancer 67:1404–1407.

LISSONI, P., S. BARNI, G. TANCINI, F. ROVELLI, A. ARDIZZOIA, A. CONTI, G.J.M. MAESTRONI (1993b) A study of the mechanisms involved in the immunostimulatory action of the pineal hormone in cancer patients. Oncology 50:399–402.

MAESTRONI, G.J.M., A. CONTI, W. PIERPAOLI (1986) Role of the pineal gland in immunity. Circadian synthesis and release of melatonin modulates the antibody response and antagonizes the immunosuppressive effect of corticosterone. J. Neuroimmunol. 13:19–30.

MAESTRONI, G.J.M., A. CONTI, W. PIERPAOLI (1988a) Pineal melatonin, its fundamental immunoregulatory role in aging and cancer. Ann. N.Y. Acad. Sci. 521:140–148.

MAESTRONI, G.J.M., A. CONTI, W. PIERPAOLI (1988b) 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., A. CONTI, W. PIERPAOLI (1989) Melatonin stress and the immune system. In: Pineal Research Reviews Vol. 7. R.J. Reiter, ed. Alan R. Liss, Inc. New York, pp. 203–226.

MAESTRONI, G.J.M., A. CONTI (1990) The pineal neurohormone melatonin stimulates activated $CD4^+$, Thy-1^+ cell to release opioid agonist(s) with immunoenhancing and antistress properties. J. Neuroimmunol. 28:167–176.

MORGAN, P.J., L.M. WILLIAMS (1989) Central melatonin receptors: Implications for a mode of action. Experientia 45:955–965.

PIERPAOLI, W., C. YI (1990) The involvement of pineal gland and melatonin in immunity and aging. J. Neuroimmunol. 27:99–109.

PIOLI, C., M.C. CAROLEO, G. NISTICO', G. DORIA (1993) Melatonin increases antigen presentation and amplifies specific and non specific signals for T-cell proliferation. Int. J. Immunopharmol. 15:463–468.

RITCHIE, A.W., L. OSWALD, H.S. MICKLEM, J.E. BOYD, R.A. ELTON, E. JAZWINSKA, K. JAMES (1983) Circadian variation of lymphocyte subpopulations: a study with monoclonal antibodies. Br. J. Med. 286:1773–1776.

RUFF, M.R., G.E. GIFFORD (1981) Tumor necrosis factor. In: Lymphokines 2. E. Pick, ed. Academic Press, New York, pp. 235–272.

VAUGHAN, M.K., G.H. HUBBARD, T.H. CHAMPNEY, G. VAUGHAN, J.C. LITTLE, R.J. REITER (1987) Splenic hypertrophy and extramedullary hematopoiesis induced in male Syrian hamsters by short photoperiod or melatonin injections and reversed by melatonin pellets or pinealectomy. Am. J. Anatomy 179:131–136.