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Mini-review

The immunoneuroendocrine role of melatonin

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Abstract: A tight, physiological link between the pineal gland and the immune system is emerging from a series of experimental studies. This link might reflect the evolutionary connection between self-recognition and reproduction. Pinealectomy or other experimental methods which inhibit melatonin synthesis and secretion induce a state of immunodepression which is counteracted by melatonin. In general, melatonin seems to have an immunoenhancing effect that is particularly apparent in immunodepressive states. The negative effect of acute stress or immunosuppressive pharmacological treatments on various immune parameters are counteracted by melatonin. It seems important to note that one of the main targets of melatonin is the thymus, i.e., the central organ of the immune system. The clinical use of melatonin as an immunotherapeutic agent seems promising in primary and secondary immunodeficiencies as well as in cancer immunotherapy. The immunoenhancing action of melatonin seems to be mediated by T-helper cell-derived opioid peptides as well as by lymphokines and, perhaps, by pituitary hormones. Melatonin-induced-immuno-opioids (MIIO) and lymphokines imply the presence of specific binding sites or melatonin receptors on cells of the immune system. On the other hand, lymphokines such as γ-interferon and interleukin-2 as well as thymic hormones can modulate the synthesis of melatonin in the pineal gland. The pineal gland might thus be viewed as the crux of a sophisticated immunoneuroendocrine network which functions as an unconscious, diffuse sensory organ.

Georges J. M. Maestroni

Center for Experimental Pathology, Istituto Cantonale di Patologia, Locarno, Switzerland

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Address reprint requests to Dr. Georges J.M. Maestroni, Center for Experimental Pathology, Istituto Cantonale di Patologia, 6604 Locarno, Switzerland.

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Introduction

In this mini-review, I will take the privilege of inserting a few biographical notes that will serve as a chronological complement to the scientific data. I suppose this may be justified by the rather keen interest this research field has aroused after our initial publications appeared.

In 1977, I was spending a 6 months stay at the Weizmann Institute of Science in Rehovot, Israel. I remember this short stay as one of the most productive and formative periods of my scientific activity. It was, in fact, during my frequent visits to the library of the Department of Chemical Immunology that I matured the idea about a possible link between the pineal gland and the immune system. At that time, while discussing with colleagues, I was often asked why should a tiny and rather mysterious organ such as the pineal gland be of any relevance to immunity. The rationale of my idea considered the evolution and function of self-identification mechanisms. I was puzzled by the observation that two huge and apparently independent systems have evolved, side by side, for the marking of self among metazoan organisms. One is the immune system with self-marking by antigen receptors at the surface of cells, and the other is self-marking by equally specific olfactants. In the case of pheromone markers a reasonable explanation is the need for identification to select a specific partner for reproduction. Immunological self-marking provided by the major histocompatibility complex (MHC) might represent an evolutionary development of the more ancient smell of self [Lewis, 1974]. In any case, self-identification is a prerequisite for both immunocompetence and sexual reproduction. It seems very improbable that two such precise and complex systems of signals and receptors have evolved for the same purpose in the same organism without being related in any way. In

search of this possible relation, I noticed that the pheromonal regulation of reproduction was shown to involve the pineal gland [Reiter, 1974]. On the other hand, evidence about any influence of the pineal gland on the immune system was rather scant and contradictory. All the available findings concerned the effect of surgical pinealectomy on organs or cells of the immune system. The results were in part controversial, with some papers claiming that absence of the pineal gland stimulated the proliferation of immunocompetent cells [Csaba et al., 1965; Bindoni and Cambria, 1968; Rella and Lapin, 1978] and others claiming the opposite [Jankovic et al., 1970; Vaughan and Reiter, 1971; Barath and Csaba, 1974]. However, most papers agreed that in pinealectomized animals the thymus undergoes a precocious involution with a profound histological disorganization [Csaba et al., 1970; Vaughan and Reiter, 1971; Barath and Csaba, 1974; Csaba and Barath, 1975]. Most authors suggested gonadal steroid hormones as possible mediators of such effects of pinealectomy. I was already working on the obvious and reciprocal relationship between the neuroendocrine and the immune system, and the results I had obtained showed a stimulation of gonadal pituitary hormones during the primary and secondary cell-mediated immune response to allogeneic cells in mice [Maestroni and Pierpaoli, 1981]. As the main function of the pineal gland was believed to concern the regulation of reproductive mechanisms, the rationale for a possible pineal involvement in immunity was further strengthened. Last but not least, the oncostatic effect of the pineal gland was already reported in many early publications [reviewed by Blask, 1984], and this also suggested a possible involvement of the immune system.

The immunoregulatory role of melatonin

In 1979 I performed the first, preliminary experiments to study the role of melatonin in immunity at the University of Zürich, Switzerland. The study was aimed at investigating whether inhibition of melatonin synthesis and secretion has any immunological consequence in mice. Inhibition of melatonin synthesis was obtained by a functional and a pharmacological approach. Melatonin is synthesized and secreted by the pineal gland upon the nocturnal (darkness) postsynaptic activation of β-adrenergic receptors [Deguchi and Axelrod, 1973] and it can be defined as the biochemical messenger of darkness [Reiter, 1991]. In fact, light prevents the adrenergic activation of the pineal, resulting in an inhibition of melatonin synthesis. In my experimental design, the choice of mice as the animal model was secondary to the decision of evaluating immunological parameters. This somehow hindered the surgical approach which was discarded, at such a preliminary stage. The results obtained indicated that mice kept under constant environmental lighting or mice treated with the β-adrenergic blocker propranolol, i.e., mice in which the synthesis of melatonin was functionally or pharmacologically inhibited, showed a depressed ability to mount a primary antibody response and a reduced spleen and thymus cellularity [Maestroni and Pierpaoli, 1981].

Effect of melatonin on acquired immunity

After this preliminary evidence of a possible immunoregulatory effect of melatonin, I had to wait until 1984 before being able to undertake a more complete and systematic study. At that time, I was already in Locarno where I met Dr. A. Conti, a young scientist who was doing his pre-doctoral work in a completely different field, but who was immediately captured by the mysteries of the pineal gland. Dr. Conti has been, and still is, the main collaborator and co-author of this fascinating melatonin-immunity story.

In those early studies, we found that both the primary antibody response to T-dependent antigens and the important autologous mixed lymphocyte reaction were depressed by evening but not by morning administration of the β-adrenergic antagonist propranolol or by a pre-treatment with p-chlorophenylalanine, a blocker of serotonin synthesis. Evening supplementation of melatonin reversed the depression of the immune responses induced by these pharmacologic interventions [Maestroni et al., 1986, 1987a]. To our knowledge, these results constituted the first evidence suggesting a possible melatonin involvement in the immuno-neuroendocrine network. Later, other laboratories and ourselves have confirmed and extended this finding. For example, pinealectomized C57BL/6 mice were shown to have a depressed ability to mount humoral responses against sheep red blood cells (SRBC) and a disturbed circadian rhythmicity (Becker et al., 1988]. In another report, inhibition of endogenous melatonin by the β-adrenergic blocker propranolol injected into hamsters, produced a decrease of spleen weight and reduced blastogenesis induced by the T cell mitogen concanavalin A. Melatonin administration counteracted the effect of propranolol bringing spleen weight and blastogenesis to values close to that shown in animals kept under short photoperiod [Champney and McMurray, 1991].

We continued the investigation, looking at possible immunopotentiating effects of melatonin administration in normal animals, i.e., in presence of a normal endogenous melatonin production. The results obtained revealed that melatonin could indeed augment the primary and secondary antibody response to SRBC [Maestroni et al., 1987b, 1988a]. This effect was exerted only when melatonin was injected daily in the late afternoon at a dosage ranging from 0.01 to 10 mg/kg body weight (b.w.). The same doses administered in the morning were ineffective and doses as high as 200 mg/kg b.w. proved to be immunosuppressive [Maestroni et al., 1987b]. A similar potentiating effect was exerted by daily melatonin administration on the secondary but not the primary cytotoxic T cell response against inoculations of Vaccinia virus [Maestroni et al., 1988a]. However, these interesting immunologic effects of melatonin appeared to be dependent on the state of immunologic activation. In other words, melatonin was able to enhance the immune response only when administered during the course of the immune response itself.

Administration of melatonin before antigen injection did not influence either the antibody production or the cytotoxic T cells response [Maestroni et al., 1986, 1987a, 1988a]. In line was this finding, it has been recently demonstrated that melatonin exaggerates collagen-induced arthritis in mice [Hansson et al., 1992]. The immunoenhancing effect of melatonin was most evident when the immune-reactivity was depressed either by acute stress or by various pharmacological treatments. In fact, daily administration of melatonin (1 μg/mouse) in the afternoon was able to counteract the effect of acute restraint stress and/or of pharmacologic immunosuppressive treatments by corticosteroids or cyclophosphamide [Maestroni et al., 1986, 1987b, 1988b, 1989]. Melatonin was effective also in restoring resistance mechanisms against a sublethal infection with encephalomyocarditis virus in mice that were exposed to acute restraint stress [Maestroni et al., 1988b]. More recently, these findings have been confirmed in aging mice and in young mice immunosuppressed by cyclophosphamide. Melatonin administration restored T-helper cells activity and interleukin-2 (IL-2) production [Caroleo et al., 1992].

In general, the immunoenhancing and anti-stress action of melatonin appeared to be restricted to responses against T-dependent antigens, i.e., to immune reactions involving T lymphocytes [Maestroni et al., 1987a,b, 1988a,b, 1989]. Furthermore, as for other biologic effects, melatonin was active only when injected in the afternoon [Maestroni et al., 1987b]. In addition, we observed that when added in vitro, in immunologic assays that were relevant to its effect in vivo, melatonin was completely ineffective.

On the basis of several reports implicating endogenous opioid peptides in immunoregulation [Sibinga and Goldstein, 1988] and of the observation that melatonin has anticonvulsant and analgesic properties in mice [Lakin et al., 1981; Kumar et al., 1982], we took into consideration endogenous opioids as possible mediators of the immunologic action of melatonin. We found that the specific opioid antagonist naltrexone was able to abolish all the immuno-enhancing and anti-stress effects of melatonin [Maestroni et al., 1987b, 1988a,b, 1989]. Furthermore, we were able to mimic the immunologic effects of melatonin by using known opioid peptides such as dynorphin 1-13 and β-endorphin [Maestroni and Conti, 1989]. This suggested the possibility that melatonin could stimulate activated immunocompetent cells to release opioid peptides. We found that physiological concentrations of melatonin can stimulate the release of opioid peptides by activated T-helper lymphocytes. These melatonin-induced-immuno-opioids (MIIO) mediated the immunoenhancing and anti-stress effects of melatonin and cross-reacted immunologically with anti-β-endorphin and anti-met-enkephalin antisera [Maestroni and Conti, 1990, 1991a]. On the other hand, MIIO seems to belong to at least two families of molecules because, when the relevant antisera were injected alone in immunodepressed, primed mice, anti-β-endorphin prevented the recovery of thymus cellularity, while anti-met-enkephalin affected the primary antibody response. However, in surgically pinealectomized mice, i.e., in a condition of melatonin depletion, neither the thymic cellularity nor the primary antibody response was influenced [Maestroni and Conti, 1991a]. These results indicated that MIIO are indeed the physiological mediators of the immunoenhancing and anti-stress effects of melatonin observed in mice.

Effect on natural immunity

As stated above, in our models we did not find any immunologic effect of melatonin in absence of antigen stimulation. However, other groups reported that melatonin has a number of effects on natural immunity. Natural killer (NK) activity has been reported to be either stimulated or depressed by melatonin treatment [Angeli et al., 1988; Del-Gobbo et al., 1989; Lewinski et al., 1989], while IL-2 production [Del Gobbo et al., 1989, Caroleo et al., 1992], antibody-dependent cellular citotoxicity

(ADCC) [Giordano and Palermo, 1991], lymphocyte blastogenesis [Champney and McMurray, 1991; Fraschini et al., 1990], and T helper/T suppressor ratio were stimulated [Lissoni et al., 1987]. It should be noted that, apparently, melatonin exerted a stimulatory activity in vivo in rodents and humans and an inhibitory one in vitro.

We investigated further the antigen-independent immunologic properties of melatonin, evaluating the effect of oral melatonin (10 mg/day) in 20 healthy volunteers on the NK and natural cytotoxicity (NC) activity of peripheral blood mononuclear cells against herpes-simplex-infected target cells and on the IL-2-induced generation of lymphokine-activated killer (LAK) cells. The results were in contrast with the above mentioned reports in that there was no effect of melatonin on these natural immune parameters [Maestroni and Conti, 1991b]. However, a number of technical differences related to the different doses of melatonin and targets used might explain the differences. Most recently, a double-blinded investigation has been conducted in collaboration with Prof. Utermohlen's group at Cornell University, Ithaca, NY, U.S.A. Normal young volunteers were treated for 10 days either with oral melatonin (10 mg) or with placebo in the evening. Before and after treatment, the concentration of salivary and serum IgA was determined in addition to other endocrine and psychological parameters. The results of this study will be reported in detail elsewhere; however, thus far, salivary IgA was increased significantly by melatonin. This result seems important, especially in view of the well known fact that salivary IgA influences susceptibility to upper respiratory infections.

In conclusion, melatonin seems to possess interesting immunoregulatory properties either in presence or in absence of an antigenic activation.

Effect on lymphokines

Gamma-interferon (γ-IFN) and IL-2 are secreted by antigen activated T-helper lymphocytes, i.e., by the same cell type that we found to be the target of melatonin. Consistently, the secretion of IL-2 and some γ-IFN-mediated immunological effects have been reported to be influenced by melatonin [Angeli et al., 1988; DelGobbo et al., 1989; Caroleo et al., 1992]. Both lymphokines are, in fact, well known stimulators of NK activity and/or other natural immune parameters. It is thus possible that these lymphokines mediate the observed effects of melatonin on such natural immune parameters. This would imply that these effects of melatonin also depend on the amount of antigen activated T-helper cells. In fact, a low number of antigen activated cells are normally present in an organism even without an overt infection. In non-pathogen-free conditions such as those in which the animals were used for the experiments, their number depends obviously on contingent and unpredictable environmental variables that may thus cause the reported variability of the melatonin action.

Relevant to these possibilities is a recent report showing that physiological concentrations of melatonin can stimulate γ-IFN production from activated human lymphocytes and that naltrexone counteracts the effect of melatonin [Muscettola et al., 1992]. This would suggest that MIIO are also involved in the effect of melatonin on γ-IFN production. On the other hand, other reports showed that γ-IFN is able to modulate either directly, in pinealocytes, or indirectly, via neural mechanisms, the synthesis of melatonin in the pineal gland [Withyachumnarnkul et al., 1990]. These interesting findings suggest the existence of a bidirectional physiological connection between products of activated immunocompetent cells and melatonin.

A negative influence of a thymic hormone on melatonin synthesis in man [Lissoni et al., 1988] has been reported and, most recently, we found that IL-2 infusion abolishes the nocturnal melatonin rhythm in humans [Lissoni et al., 1990].

Apart from the above mentioned considerations about a possible effect of melatonin on natural immune cytolytic effectors via lymphokines, various hormones have been reported to influence such effectors. In connection with melatonin, the most relevant are opioid peptides, growth hormone (GH), and prolactin (PRL). Central nervous system opioid peptides have been, in fact, implicated either to explain some melatonin effects or as central modulators of NK activity [Shavit, 1991]. On the other hand, both GH and PRL have been reported to increase NK activity and also to be influenced by melatonin [Kelley, 1991; Bernton et al., 1991]. The pineal–immune system interactions and their functional effects are schematized in Figure 1.

Melatonin as an immunotherapeutic agent

The use of melatonin as immunotherapeutic agent seems straightforward, especially in states of immunodeficiency, a condition in which melatonin was shown experimentally to be most active. Unfortunately, acquired-immunodeficiency-syndrome (AIDS) is a most dramatic human model of immunodeficiency. We have performed a preliminary study treating 11 HIV-infected patients with melatonin at a different stage of the disease (6 LAS-ARC

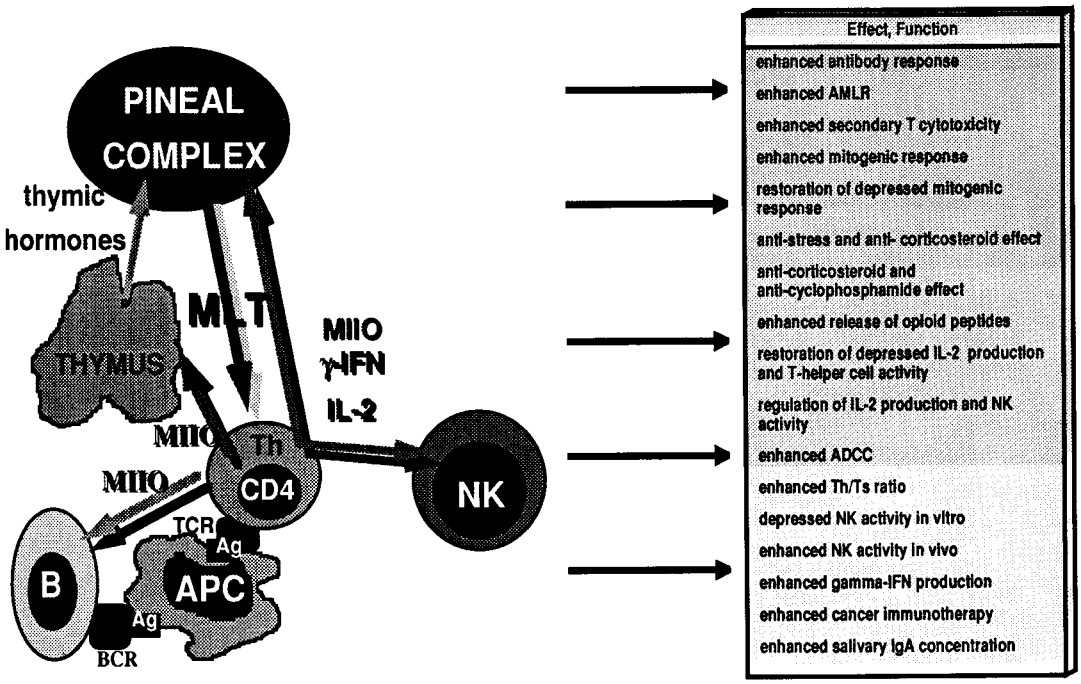

Fig. 1. The melatonin-immune system network is shown together with its known functional correlates. MLT: melatonin; MIIO: melatonin-induced-immuno-opioids; γ-IFN: gamma interferon; IL-2: interleukin-2; CD4 Th: T helper cell; TCR: T cell receptor; BCR: B cell receptor; APC: antigen-presenting cell; Ag: antigen; B: B lymphocyte; NK: natural killer cell.

and 5 AIDS patients). Melatonin was given orally at a dose of 20 mg/day in the evening for 56–84 consecutive days, but its immunological effects were rather erratic. The exception was a general increase of the peripheral blood mononuclear cell number and response to the T cell mitogen PHA [Maestroni, G.J.M., Conti, A. and DeLalla, F., unpublished data]. However, the low number of patients involved and the rather advanced stage of the disease did not allow any definite conclusion. Nevertheless, no matter which parameter was considered, melatonin appeared to act following a bell-shaped kinetic curve with a peak after 14–28 days of treatment [Maestroni, G.J.M., Conti, A. and DeLalla, F., unpublished data]. This observation suggested that melatonin should be administered periodically with washout intervals. Accordingly, the protocol we then used and are still using for all clinical studies consists of treatments of 21–28 consecutive days, interposed by a one week washout interval. Of course, the time-dependency of melatonin treatments is a very crucial issue and deserves to be elucidated. In any case, I think the use of melatonin in HIV-positive patients should be seriously considered.

We also feel cancer immunotherapy may be one of the most promising and relevant approaches in which melatonin may be used therapeutically. Cancer has been often associated to depressed melatonin secretion and immune reactivity [Ader, 1981; Blask, 1984]. Recent strategies in cancer immunotherapy are centered around the activation of natural cytolytic mechanisms. NK and LAK cells lyse malignant or virus-infected cells while sparing normal cells [Ortaldo and Heberman, 1984]. The interesting finding that IL-2 can potentiate NK activity and generate LAK cells from NK and T cells has triggered experimental and clinical trials with encouraging results [Rosenberg and Terry, 1987]. Unfortunately, the anti-cancer action of IL-2 is apparent only at high concentrations and its severe toxicity limits its use [Rosenberg and Terry, 1987]. The IL-2 dependent LAK phenomenon might, however, reflect a physiologic mechanism that, in addition to the better known NK activity, would constitute the most basic anti-cancer weapon that the organism may use. If this were true, the concentrations of IL-2 involved would certainly be non-toxic or, alternatively, only locally elevated, for example within a lymphnode microenvironment. In addition, other microenvironmental components might synergize with IL-2 and here the neuroendocrine system might well play an important role.

On the basis of the findings reported above, melatonin appeared to be a good candidate for

combination with IL-2 in the immunotherapy of cancer. In addition, a large body of evidence points to the pineal gland and melatonin as powerful oncostatic effectors [Blask, 1984]. Therefore, we performed experiments to evaluate the effect of a combined melatonin + IL-2 treatment on the development and growth of established pulmonary metastases. The results of these experiments showed that melatonin may indeed potentiate the IL-2 anti-cancer action in an additive fashion [Maestroni and Conti, 1993]. As expected, melatonin alone also exerted a significant antitumor effect. However, the decrease of lung colonies did not apparently depend on a melatonin-dependent systemic stimulation of NK or LAK cells activity. Other more subtle or local mechanisms might be involved. For example, besides a direct influence on tumor cells, melatonin action on macrophages or on ADCC activity might explain the effect observed. At any rate, melatonin seems to allow a reduction of the effective IL-2 dose with evident practical implications.

In collaboration with Dr. P. Lissoni, Divisione di Radioterapia Oncologica, Ospedale S. Gerardo, Monza, Italy, we are conducting some clinical trials on the basis of our animal findings. The schedule of melatonin treatment consists of a daily oral administration of 10–50 mg melatonin at 2000 hr, starting 7 days before the onset of IL-2 therapy. Melatonin was administered chronically for 3–4 weeks with washout intervals of 1 week. Tumor histotypes, in which a concomitant treatment with IL-2 and melatonin is tested, include metastatic renal cancer, metastatic non-small cell lung cancer, metastatic colorectal carcinoma, metastatic hepatoma, metastatic gastric carcinoma, and mammary carcinoma. At present, the results obtained are preliminary due to the small number of patients; however, they indicate that melatonin may indeed be successfully combined with IL-2 in cancer immunotherapy.

Another study concerned cluster headache (CH). CH is perhaps the most painful and severe type of primary headache. A significant decrease in nocturnal melatonin levels, together with an impairment of immune parameters, has been described in CH patients [Waldenling et al., 1987; Martelletti et al., 1987]. Nine male CH patients were treated with 5 mg of oral melatonin at 2000 hr for 10–60 days. Ten days after melatonin therapy, the NK lytic units in peripheral blood mononuclear cells increased significantly while total pain index showed a moderate decrease in one patient, no variation in 3 patients, and a marked improvement in 5 other patients [Maestroni and Conti, 1993]. Interestingly, the basal nocturnal concentration of serum melatonin was lower in responders than in non-responders. These results might be interpreted on the basis of the immunoenhancing effect of melatonin in addition to its immuno-opioid-inducing action.

Anticipated research in the next decade

Basic studies

No doubt the emergent immunoregulatory role of melatonin deserves to be studied further. First of all, the influence of melatonin on the cyto-lymphokines cascade and the nature of the immunocompetent cells involved are far from clear. Being lymphocyte products acting on immunocompetent cells, MIIO can also be considered as lymphokines. In case of murine lymphocytes, we have characterized such substances by their immunological crossreactivity as immunoreactive β-endorphin and/or met-enkephalin. However, a biochemical purification and molecular identification of murine and human MIIO will be needed for a correct understanding of their function. MIIO seems to have the specific function of counteracting the effect of corticosteroids whose serum concentration may be elevated via the hypothalamo-pituitary-adrenal response to stress events and/or antigen activation. It has been suggested that the elevation of adrenal steroids associated with the immune response has the function of controlling the clonal expansion of immunocompetent cells with low antigen affinity. This would serve to diminish the probability of both autoimmune and lymphoproliferative diseases [Besedovsky et al., 1986].

In relation to lymphoproliferative diseases, we have shown that MIIO do accelerate the development of RadLV-induced T-cell leukemia in mice [Conti et al., 1992]. With regard to autoimmunity, besides the immunoenhancing properties of MIIO, it seems reasonable to hypothesize that the intrathymic maturational events leading to self/non-self recognition might be related to the functional effect of MIIO binding to specific sites in the thymus. This would be relevant not only for autoimmune diseases but also, in general, for the overall immune homeostasis. Epidemiological and experimental investigations in this direction are clealry needed. These investigations will be but one side of the coin of the pathophysiological significance of the melatonin-immune system network. We have some preliminary evidence that the immunoregulatory action of melatonin and MIIO production may be influenced by factors such as age and sex. Again, this might be related to the physiological development-involution of immunological and thymic functions and to the etiopathogenesis of immune-based diseases. In addition, most recently it has been reported that the

reproductive effects of melatonin depend on the interaction of steroid hormones and opioid peptides in the central nervous system [Bittman, 1992]. It might thus be possible that the melatonin-immuno-opioid relationship is mirroring similar connections in the central nervous system. In any case, this point deserves to be investigated.

Besides these basic but clinically-oriented studies, a more fundamental approach is clearly also needed. A relevant issue concerns the presence and characterization of melatonin receptors in cells and organs of the immune system. A recent report described the specific binding of ^{125}I-melatonin in membranes of the spleen from pigeons, ducks, quail, chickens, mice, and guinea pigs [Pang et al., 1991]. In regard to opioid binding in peripheral immunocompetent cells, the evidence is much clearer and established [Sibinga and Goldstein, 1988]. However, our results imply also the presence of opioid binding sites in the thymus gland. We have reported the presence of specific opioid binding sites in the thymus [Maestroni and Conti, 1991a] and, perhaps, one should also search for thymic melatonin binding sites. It seems clear that such studies will be fundamental for our understanding of the immunoregulatory role of melatonin. Furthermore, they will constitute the basis for molecular studies that should investigate signal transduction mechanisms. These studies will, obviously, use immunocompetent cells as a target of melatonin. Because of their ready availability, such melatonin targets might provide key information related to the general physiological function(s) of melatonin in man.

Clinical studies

To date, we can reasonably claim that melatonin is an antineoplastic and an immunoenhancing agent. In addition, it is widely recognized that melatonin is a non-toxic substance that can be safely administered orally to patients. However, if melatonin is to be developed as an immunostimulating and oncostatic drug, it is necessary to investigate whether its effects on the immune system or tumor growth are season-dependent, as has been done for the reproductive system. On this basis, 2 *most urgent and important* studies should be performed. The first investigation should evaluate a possible preventive effect of chronic, *periodic* melatonin treatment in patients who are at high risk of developing primary and/or secondary cancer. In view of its high incidence and our current good knowledge of its risk factors and hormonal dependence, mammary cancer should have the highest priority in such studies. However, other primary and secondary neoplasias should also be considered. For example, many chemo- and radiotherapeutic anticancer regimens involve a high risk for secondary cancers and, in general, do not favor a good immunological reactivity that in these cases is a very important, but often neglected, defense mechanism.

The second urgent study is more related to immunology. Chronic, *periodic* melatonin treatment should be evaluated for its potential benefit in asymptomatic, HIV-positive individuals. Here one should consider that both HIV and melatonin seem to have the same target, i.e., CD4+, T lymphocytes, and thus melatonin might be eventually helpful before the development of AIDS, i.e., in presence of a normal or sustained concentration of CD4+, T lymphocytes. These large and long-term studies should be multicentered and possibly supported by pharmaceutical concerns or by international organizations. Apart from prevention of cancer and/or AIDS, which I consider among the real possibilities of a pharmacological melatonin treatment, other immunodeficiencies or immune-based diseases might benefit from such an approach; included in this group would be states of immunological weakness secondary to viral infections, acute and/or chronic stress events, surgical interventions, aging etc. However, the relevance of a disturbed endogenous melatonin producton and its possible influence on the immune reactivity and/or immune-based diseases remains to be elucidated. In other words, in the human it is still not known whether a sustained derangement of melatonin synthesis and/or release is causative of any immunological abnormality. To answer this question, one should select among pathophysiological situations, environmental conditions, or pharmacological treatments producing as a main consequence an alteration of melatonin synthesis. Shift work, frequent transcontinental flights, chronic exposure to electromagnetic fields, treatment with β-adrenergic blockers, and pinealectomy are some examples of conditions that should be investigated. Such investigations should focus on functional aspects of the immune system and not only on the phenotypic characterization of immunocompetent cells. In addition, any study of this type should take in account not only melatonin per se, but also its immuno-opioids mediators (MIIO) and their possible function. Furthermore, the risk for cancer and/or immune-based diseases should be assessed in individuals with early blindness. Last but not least, an investigation that would directly challenge my original rationale of the pineal gland as physiological expression of the relation between the immune

system and the olfactory system should concern the epidemiological correlates of partial anosmia. Olfactory blindness to certain odors is a rather common clinical condition that might be associated with increased melatonin production and might, thus, reveal unexpected epidemiological associations.

Concluding remarks

The immunoregulatory properties of melatonin and the pineal sensitivity to substances of immunologic origin such as cytokines or thymic hormones suggest the existence of a bidirectional flow of information between the pineal gland and the immune system. I like to think of such sophisticated interactions as a pineal-immune sensory organ. Environmental threats such as photoperiod variations, microbial pathogens, and/or other stressors are sensed by different parts of the same diffuse sensorial organ and the information is integrated and elaborated to give the appropriate response. A virus detected by the immune system will produce an acute activation of immunocompetent cells which may finally modulate the pineal melatonin response to a given photoperiod which, in turn, will influence the specific immune response along with other melatonin-sensitive neuroendocrine mechanisms.

On the other hand, a robust melatonin rhythm might prevent infectious events and malignant proliferation. This would result in a decreased frequency of specific and acute activation of the immune system which is perceived as a stress event due to its neuroendocrine correlates. In this case, melatonin would be involved via the release of MIIO or of lymphokines that can counteract the adverse immunological effects of such an event both at central (thymus) and peripheral level. It seems thus reasonable to conceive the pineal gland as the crux of a sophisticated network whose derangement or incorrect functioning may have several negative consequences. Disturbances of melatonin-sensitive neuroendocrine mechanisms might be seen as indirect immunologic perturbations and immunodepressive states as consequences of primary psychoneuroendocrine disorders. The neuroendocrine and immune correlates of emotional disorders, chronic psychogenic and/or environmental stress, psychiatric diseases, dementia, cancer, and aging might depend on such mechanisms. In conclusion, the emerging picture documents the existence of an important new physiological link between the neuroendocrine system and the immune system. Such evidence opens interesting possibilities to improve our understanding and perhaps control of a variety of serious diseases.

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