Rheumatoid arthritis

The melatonin-cytokine connection in rheumatoid arthritis

M Cutolo, G J M Maestroni

Melatonin up regulates cytokine production and immune function

A substance from pineal gland extracts, which lightens the skin melanocytes of amphibians and fishes, was isolated in 1958 and called melatonin (MLT, *N*-acetyl-5-methoxytryptamine).[1] In both diurnal and nocturnal species, the absence of light at night stimulates MLT biosynthesis. Electrical signals originating from the retina reach the suprachiasmatic nuclei which, in turn, send inputs via the paraventricular nuclei to the spinal cord and then to the superior cervical ganglia. The fibres terminate at the pinealocytes.[2] Absence of light results in increased norepinephrine release and activation of α1 and β-adrenergic receptors on the pinealocytes. This triggers a series of intracellular responses, resulting in activation of the enzymes *N*-acetyltransferase (EC 2.3.1.87) and hydroxyindole-*O*-methyl transferase (EC 2.1.1.4), which convert serotonin into MLT.[2] The circadian nocturnal release of MLT has a profound influence on the internal environment of the organism, with diverse physiological effects. The main function of MLT seems to be that of synchronising the organism in the photoperiod and it may have a role in reproduction, metabolism, seasonality, thermoregulation, and immunity.

MELATONIN AND THE HUMAN IMMUNE SYSTEM

Animal studies have shown that binding of MLT to specific receptors in antigen activated T helper (Th) cells results in an up regulation of cytokine production and immune function.[3] In general, the immunoenhancing action of MLT seems restricted to T dependent antigens and to be most pronounced in immunodepressed situations. For example, MLT may completely counteract thymus involution and the immunological depression induced by stress or glucocorticoid treatment,[4] or restore depressed immunological functions after soft tissue trauma and haemorrhagic shock.[5] MLT may also rescue haematopoiesis in mice treated with cancer chemotherapeutic compounds.[6] This effect apparently involves the endogenous release of granulocyte/macrophage colony stimulating factor and MLT-induced opioid cytokines.[3 6 7]

The immunoenhancing action of MLT has been confirmed and extended in a variety of animal species and in humans and in birds.[8–12] In human peripheral blood mononuclear cells, at physiological concentrations MLT has been reported to stimulate the production of interleukin 1 (IL1), IL2, interferon γ (IFNγ), IL6, and IL12 but not IL4.[13–15] Physiologically, the nocturnal MLT peak has been associated with a high IFNγ/IL10 ratio—that is, the MLT rhythm positively correlates with the rhythmicity of the Th1/Th2 cell ratio.[16] In patients with ischaemic stroke an impaired nocturnal MLT excretion has been associated with impaired cell mediated immunity and changes of lymphocyte subsets.[17] Most interestingly, reduction of MLT secretion has been reported to parallel disease progression and to correlate with serum IL12 levels in HIV-1 infected patients.[18] These human studies confirmed that MLT possesses important immunoenhancing properties and suggest that MLT may favour a Th1 cell response.

> ''MLT possesses important immunoenhancing properties''

Most recently, the connection between IL2 and MLT in human lymphocytes has been strengthened by the observation that MLT may be synthesised and released in large quantity by human lymphocytes.[19 20] The lymphocyte MLT production seems to be strictly linked to the release of IL2 as inhibition of MLT synthesis resulted in a decrease of IL2 production and addition of exogenous MLT resulted in an increase of IL2 production. These findings indicate that in addition to pineal gland, human lymphoid cells are an important physiological source of melatonin and that this melatonin might play a part in the regulation of the human immune system, possibly by acting as an intracrine, autocrine, and/or paracrine substance.[13 14 19 20] The MLT/IL2 connection seems particularly relevant because IL2 has a central role in immune homoeostasis. Recent studies indicate that a failure in the production of CD4+CD25+ regulatory T cells is the underlying cause of autoimmunity in the absence of IL2.[21] Yet, IL2 has been used clinically to enhance T cell immunity in patients with AIDS or cancer, and blocking antibodies to the IL2 receptor are used to inhibit T cell responses against transplanted tissues.[22] In analogy to IL2, MLT has also been used to enhance T cell immunity in patients with cancer.[23] Perhaps, the ability of MLT to enhance production of inflammatory cytokines from human monocytes/macrophages, including IL12,[24–26] turns the MLT/IL2 connection towards the enhancement of T cell immunity rather than to formation of T regulatory cells.

MLT, IL2, AND GLUCOCORTICOID RESISTANCE

Endogenous glucocorticoids, the end-effectors of the hypothalamus-pituitary-adrenal (HPA) axis, also have an important role in regulating homoeostasis of the immune system.[27] It is generally recognised that glucocorticoids suppress peripheral Th cell responses.[28] However, the effect of glucocorticoids on T cell functions may depend on the activation and differentiation state of T cells, as well as on the conditions or agents used for stimulation.[28] Thus, resting T cells appear to differ from activated T cells in their response to glucocorticoids, while infections, cytokines, and proinflammatory factors can alter the sensitivity of T cells.[29]

In clinical practice, reduced T cell sensitivity to the suppressive action of glucocorticoids is a common problem that complicates the management of chronic inflammatory and autoimmune diseases. Patients with glucocorticoid resistance usually have severe inflammation, associated with significantly high levels of cytokines, growth factors, and costimulatory factors in the inflamed tissues.[30–32] In particular, it has been reported that IL2 may induce glucocorticoid resistance in Th cells.[33] As mentioned above, MLT may counteract the immunosuppressive effect of endogenous or exogenous glucocorticoids.[34] The mechanism of this effect was related to stimulation of opioid cytokines in T lymphocytes and/or to a direct inhibition of glucocorticoid receptor expression.[35 36] In addition to these effects, the MLT/IL2 connection suggests that excessive MLT production might be involved in glucocorticoid resistance. This hypothesis should be tested.

MLT, CYTOKINES, CIRCADIAN RHYTHMS, AND RA

In 2002 our first study evaluated MLT levels in patients who had rheumatoid arthritis (RA), with a focus upon analyses of circadian variations.[37] MLT serum levels at 8 pm and 8 am were found to be significantly higher in patients who had RA than in healthy controls ($p<0.05$). The differences were more evident in patients who were older than 60 years.

> ''MLT serum levels are significantly higher in patients with RA than in controls''

In patients who had RA and in healthy subjects, MLT levels increased progressively from 8 pm to the early morning hours; however, they reached peak levels at midnight in patients who had RA, which was at least 2 hours earlier than in controls. Subsequently, MLT concentrations in patients who had RA reached a plateau that lasted for 2–3 hours; this was not observed in controls. After 2 am, MLT levels decreased similarly in patients who had RA and in healthy subjects. The study confirmed that the nocturnal rhythm of MLT occurs also in patients who have RA, but with an earlier peak and a longer duration in the early morning.[37] IFNγ, IL1, IL6, tumour necrosis factor α (TNFα), IL2, and IL12 production (Th1 cytokines/promoting cytokines) reach their peak during the night and early morning, at the same time that MLT serum levels are highest and plasma cortisol is lowest.

Accordingly, among the signs of joint inflammation in patients who have RA, the intensity of pain varies as a function of the hours of the day; pain is greater after awakening in the morning than in the afternoon or evening.[38] Circadian changes also are observed in joint swelling and finger size in the early morning in patients affected by RA.[38] Therefore, MLT may activate the inflammatory response during the night, at least in RA, which is considered to be a Th1 cytokine driven immune disease.[39]

We found that MLT was detectable in high concentration in synovial fluids from patients who had RA, and binding sites for MLT were present in synovial macrophages.[40 41] In addition, cultured RA synovial macrophages respond to MLT stimulation with an increased proinflammatory cytokine production.[24]

Interestingly, recent studies and reviews examined the epidemiological evidence which suggests that ultraviolet radiation may have a protective role in RA. A gradient of increasing incidence of RA with latitude and seasonal variation has also been reported[42–44] Therefore, we evaluated serum MLT, cortisol, TNFα, and IL6 circadian rhythm in patients who had RA from a northern European country (Estonia).

Furthermore, we compared the MLT and cortisol levels in that group with patients from a southern European country (Italy), to detect a possible influence of different daily winter photoperiods.[45 46] Blood samples were obtained during the months of January and February. The study showed that, a significantly higher MLT concentration ($p<0.01$) and an earlier peak was seen in Estonian patients who had RA than in controls matched for age and sex. In addition, MLT serum concentrations were significantly higher in Estonian patients who had RA than in the Italian patients, at midnight, and the difference was even greater over the study duration. No significant difference was seen in serum cortisol levels between Estonian patients who had RA and their healthy controls.[45 46]

Significantly higher serum IL6 and TNFα concentrations were also observed at 4 pm and midnight in Estonian patients who had RA than in the Italian patients who had RA.[45 46]

This important study shows, for the first time, that in a northern European country, the circadian serum concentrations of MLT and TNFα are significantly higher than in matched patients who had RA from a southern European country. In addition, MLT and TNFα concentrations were found to be increased in patients who had RA.

> ''Circadian serum concentrations of MLT and TNFα are significantly higher in patients with RA from northern Europe than in those from further south''

The reduced daily light exposure in northern Europe, at least during the winter, might explain the higher and more prolonged MLT serum concentrations that were found in northern patients who had RA.

Interestingly, several studies in patients with RA showed that overall activity of the HPA axis, particularly the glucocorticoid synthesis, remained inappropriately normal (or relatively low) and apparently was found to be insufficient to inhibit continuing inflammation.[47] Therefore, an imbalance between the anti-inflammatory effects of cortisol and the proinflammatory effects of MLT seems evident in patients affected by RA and supports the increased Th1 cytokine production during the night.[48]

Finally, the increased prevalence of autoimmune diseases, such as RA, which is seen in northern Europe also may be related to the increased immunostimulatory effects that are exerted during the night by MLT and to a reduced neuroendocrine modulation during the light phase of the photoperiod (cortisol). The prevalence of RA is, in fact, much higher in northern Europe than in the Mediterranean countries, with rates of 1.96% in Finland, 1.1% in England, 0.9% in Sweden, Denmark, and the Netherlands compared with 0.2% in Greece and 0.3% in Italy and Israel.[49 50]

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Authors' affiliations
M Cutolo, Research Laboratory and Division of Rheumatology, Department of Internal Medicine, University of Genova, Viale Benedetto XV,6, 16132 Genova, Italy
G J M Maestroni, Centre for Experimental Pathology, Cantonal Institute of Pathology, Via In Selva 24, PO Box, 6601 Locarno, Switzerland

Correspondence to: Professor M Cutolo, mcutolo@unige.it

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