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MELATONIN AND ITS INFLUENCE ON IMMUNE SYSTEM

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Melatonin was initially extracted from the pineal gland and was thought to be produced exclusively by this organ. Subsequently it was shown that melatonin is also produced in other tissues including the gastrointestinal tract, retina and cells of the immune system. Melatonin is believed to be an important regulator of circadian and seasonal rhythms. Over the last thirty years, a great number of reports have documented a relationship between melatonin/pineal gland and the immune system in various species, including humans. In this review, current knowledge about the role of melatonin in the regulation of immune responses will be discussed.

Key words: *melatonin, immunoregulation, inflammation, innate immune response, adaptive immune response.*

INTRODUCTION

Our bodies are constantly exposed to different microorganisms that are present in the environment. However, contact with pathogenic microorganisms rarely results in infection. This is because our bodies are protected by both innate and adaptive immune mechanisms.

The innate immune system consists of many cells, such as macrophages, dendritic cells, mast cells, neutrophils, eosinophils and natural killer (NK) cells. These become activated during inflammation, which is virtually always a sign of infection with pathogenic microbes (1). The main goal of these cells is to eliminate the infection. It is worthy to underline that innate responses depend on host recognition of highly conserved structures present on microorganisms called "pathogen-associated molecular patterns" (PAMPs) (2). PAMPs are recognized by

"pathogen recognition receptors" (PRR) (2). The two major currently recognized groups of PRRs in humans are toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-containing proteins (3). Whereas TLRs are associated with the plasma membrane or, in some case, with lysosomal and/or endosomal vesicles, both NOD1 and NOD2 are present in the cytosol (4).

However, in certain types of infection, the innate immune system is not able to deal with the infection and then an adaptive immune response is required. In such infections, the innate immune system can instruct the adaptive immune system regarding the nature of the pathogen through the expression of CD80 and CD86 costimulatory molecules on dendritic cells and by producing cytokines to direct the response.

There are two major classes of adaptive immune responses. The first, the so-called "cellular response", is mediated by MHC II restricted, Th1 $CD4^+$ T cells which drive delayed type hypersensitivity (DTH) responses, or MHC class I restricted $CD8^+$ T cells which mediate direct cytotoxicity. The cellular response is principally directed against intracellular pathogens (5). During the effector phase of DTH, Th1 lymphocytes release proinflammatory cytokines like IFN-γ, which induce local tissue cells to produce chemokines that recruit and activate an infiltrate of bone marrow-derived leukocytes (6). $CD8^+$ T cytotoxic (Tc) cells kill infected host cells *via* released perforin and granzymes and by triggering FasL dependent apoptosis.

The second type of adaptive immune response is the humoral immune response and is mediated by antibodies produced by B lymphocytes (1). In this type of immune response, B cells receive support from Th2 T cells that produce IL-4, IL-5, IL-6 and IL-13. The main function of the humoral response is to destroy extracellular microorganisms and prevent the spread of infection. The health of an organism is dependent on the ability of all of these branches of the immune system to function together to protect from and control pathogenic organisms as well as cancerous tissue. At the same time there must be mechanisms to protect the organism from developing inappropriate immune responses that are harmful to self (allergy, autoimmunity) as well as to control and resolve inflammatory responses after clearance of the pathogen. This demonstrates the importance of the balance of the immune response and its strict control by regulatory mechanisms.

DIFFERENT MECHANISMS OF IMMUNOREGULATION

The immune response is negatively regulated by the action of T suppressor (Ts) cells, which are also called T regulatory (Treg) cells. It is becoming increasingly clear that there are multiple populations of T cells with regulatory activity and that these can use different mechanisms, including direct cell-to-cell contact and production of anti-inflammatory cytokines, to dampen the immune

response (7-9). Additionally, there is a body of evidence that the nervous and endocrine systems can also interact with the immune system to modulate its function (10). Indeed, it has been shown that many neurotransmitters, neuroendocrine factors and hormones can dramatically alter immune function and that, conversely, cytokines released by immune cells can affect the central nervous system (11). It is also believed that environmental signals can regulate many immune processes in different species including humans. It has been demonstrated that light is one of the environmental signals that can modulate the immune system. Although most of the light energy received by the retina is relayed to the visual cortex for vision, an alternative pathway from the retina relays a small part to the suprachiasmatic nucleus, which is part of the hypothalamic region in the brain (12, 13). The suprachiasmatic nucleus is thought to direct circadian rhythm and therefore controls many processes in the body such as temperature, appetite, and mood (12). The pituitary and pineal glands are also involved in light-induced neuroendocrine changes. The neuroendocrine hormones that are sensitive to modifications in circadian rhythm are growth hormone, thyroid hormones, thyroid-stimulating hormone, plasma cortisol and melatonin (12, 14).

Over the last thirty years, a great number of reports have documented a relationship between melatonin from the pineal gland and the immune system in various species including humans (15, 16). Current knowledge about the role of melatonin in the regulation of immune mechanisms will be discussed further below.

THE INFLUENCE OF MELATONIN ON THE IMMUNE SYSTEM

It is important to note that melatonin is produced not only by pineal gland, but also in the retina, kidneys and digestive tract (17). This suggests that the immune system might be affected by melatonin originating from different organs of the body. Additionally it was found that human peripheral blood mononuclear cells synthesize biologically relevant amounts of melatonin (18). This indicates a potential intracrine and paracrine role of melatonin in immune regulation.

It is believed that melatonin influences cells of the immune system *via* melatonin receptors. Both membrane and nuclear melatonin receptors have been identified on leukocytes. Membrane receptors were found mostly on $CD4^+$ T lymphocytes, but also on CD8 T and B cells (19-21). Through these receptors, melatonin modulates the proliferative response of stimulated lymphocytes. On the other hand, melatonin induces cytokine production by human peripheral blood mononuclear cells via the nuclear melatonin receptor (22).

The immunoregulatory activity of melatonin was determined with the use of following experimental models: surgical or functional pinealectomy, *in vivo* treatment with melatonin or *in vitro* treatment of immune cells with melatonin. Some studies demonstrated an immunoenhancing activity for melatonin. Daily

afternoon injections of melatonin induced an increase in thymus weight in the gerbil (23) and spleen hypertrophy in the Syrian hamster (24). Treatment with melatonin also increased the mitogenic response of mouse spleen cells to concanavalin A and lipopolysaccharide (LPS) (25, 26). The mechanism by which melatonin acts to enhance the immune response is not fully understood. It is believed that, in part, it may act to increase phagocytosis and antigen presentation (20). Indeed it was shown that treatment with melatonin enhanced antigen presentation by splenic macrophages to T cells with a concurrent increase in MHC class II expression and synthesis of the proinflammatory cytokines IL-1 and TNF-β (27). Additionally, melatonin was observed to induce IL-12 production to drive T cell differentiation towards the Th1 phenotype (28). The activating effect of melatonin on the immune system is also mediated through the regulation of gene expression of cytokines in the spleen, thymus, lymph nodes and bone marrow. It was shown gene expression of M-CSF, TNF-α, TGF-β and SCF was increased in peritoneal macrophages, while IL-1β, IFN-γ, M-CSF, TNF-α and SCF was increased in spleen cells of mice treated with melatonin (29).

Other studies have shown that melatonin administration increases NK cell activity in humans (30). Similar observations were made in mice where treatment with melatonin increased antibody dependent cellular cytotoxicity (ADCC) (31, 32). Aside from activation of immune cells by melatonin, this hormone also enhances production of NK cells and monocytes in the bone marrow of mice (33). Melatonin seems also to promote the survival of precursor B cells in mouse bone marrow (34).

To summarize, melatonin is considered as a modulator of haemopoiesis and of immune cell production and function. Melatonin has been demonstrated to stimulate cytokine production, enhanced phagocytosis, increased NK cell activity and skewing of the immune response toward a helper T cell type 1 profile. The activating effect of melatonin on the immune system is presented in *Fig. 1*.

Melatonin has been shown to aggravate Th1 dependent inflammatory response in animal models of multiple sclerosis (35) and rheumatoid arthritis (36). Additionally, it was found in rats that melatonin is important in controlling cell recruitment from the bone marrow and their subsequent migration to the lung. It may suggest that melatonin is involved in allergic lung inflammation (37). This observation is in line with human studies showing that elevated serum melatonin is associated with the nocturnal worsening of asthma (38). Moreover, it is suggested that melatonin may play a role in the etiology and treatment of several dermatoses e.g. atopic eczema, psoriasis and malignant melanoma (39, 40).

Importantly, while many studies have implicated melatonin as a positive regulator of immune responses, a number of other reports have suggested that melatonin may act as an anti-inflammatory agent, inhibiting immune responses in some cases. It is believed that the anti-inflammatory action of melatonin is at least partly due to the induction of Th2 lymphocytes that produce IL-4, thereby inhibiting the function of Th1 cells (41). Indeed, melatonin has been shown to be

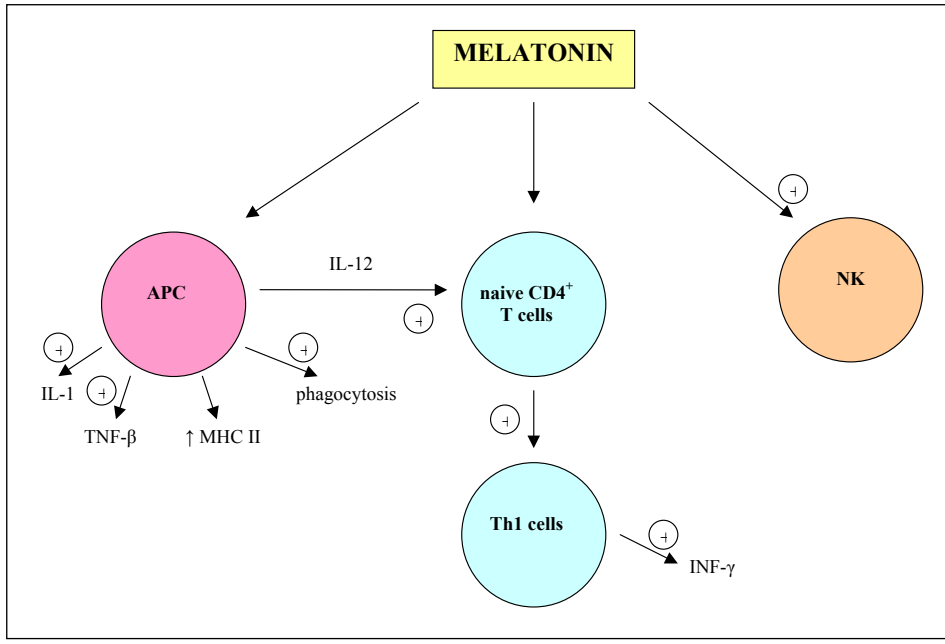

Fig. 1. The activating effect of melatonin on the immune system.
Melatonin activates both innate (antigen presenting cells (APC), natural killer (NK) cells) and adaptive immune responses ($CD4^+$ T lymphocytes).

protective in septic shock (42), an animal model of ulcerative colitis (43) and experimental pancreatitis (44, 45).

MELATONIN AND INFLAMMATION

Inflammation begins when cells within the infected tissue, whether they be epithelial or stromal cells, tissue resident mast cells or dendritic cells, recognize an inflammatory stimulus. These signals lead to the recruitment and activation of effector cells of the immune system. As mentioned in Introduction, PRR (*e.g.* TLR and NOD) play a crucial role in sensing microbial invaders by recognition of PAMPS (46, 47). PRR ligation leads to the transcription of nuclear factor-kappa B (NF-κB)-dependent genes, many of which encode for proinflammatory cytokines and chemokines (48). Additionally, recognition of PAMPS by PRR results in NF-κB-dependent expression of defensins that possess strong bactericidal activity (49). NF-κB is also important for the synthesis of the enzymes that generate prostaglandins and reactive oxygen species (*e.g.* COX and iNOS), substances that are also involved in inflammation (48). Furthermore, the expression of adhesion molecules on circulating leukocytes and endothelium involved in leukocyte migration are also regulated by NF-κB (50, 51).

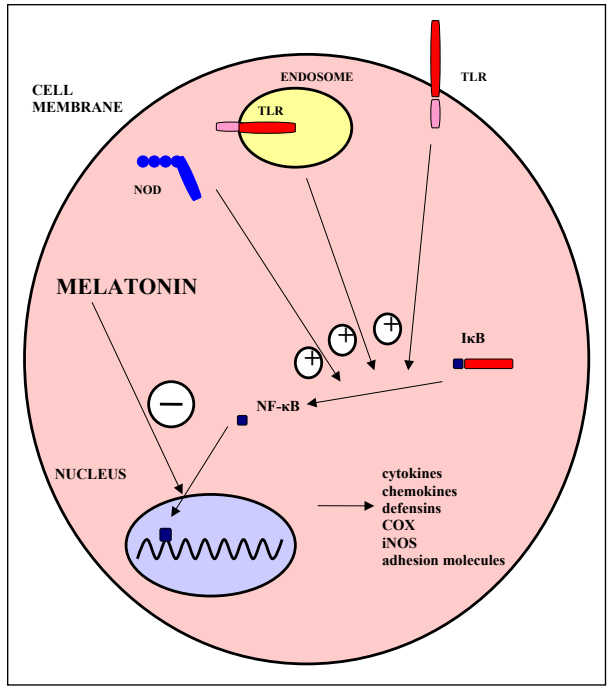

Fig. 2. The anti-inflammatory effect of melatonin.
Melatonin inhibits NF-κB binding to DNA and prevents its translocation to the nucleus. This, in turn, reduces the production of proinflammatory cytokines and chemokines. Additionally, melatonin inhibits expression of adhesion molecules and suppresses synthesis of the enzymes that generate prostaglandins and reactive oxygen species (*e.g.* COX and iNOS).

Because NF-κB regulates a large number of genes involved in the immune response and inflammation, this pathway is a likely target to silence chronic inflammation that occurs in various diseases *e.g.* autoimmunity. Recently, melatonin has been found to reduce NF-κB binding to DNA, probably by preventing its translocation to the nucleus (52). This, in turn, reduced the production of proinflammatory cytokines and chemokines. Additionally, because melatonin has been shown to reduce adhesion of leukocytes to endothelium as well as transendothelial migration, it may also suppress the expression of NF-κB-regulated adhesion molecules (53). Finally, melatonin has been shown to reduce recruitment of neutrophils to the site of inflammation (54, 55). The anti-inflammatory effect of melatonin is presented in *Fig. 2*.

Septic shock caused by systemic bacterial infection is a form of uncontrolled acute inflammatory response. This syndrome is characterized by hypotension, inadequate perfusion, vascular damage and disseminated intravascular coagulation leading to multiple organ failure and death (56). It is known that many of the pathological symptoms of septic shock to Gram-negative bacteria are attributable to (LPS) present in bacterial membranes. Nitric oxide (NO) produced in response to LPS has been shown to be responsible for LPS-induced hypotension and vascular hyporesponsiveness, suggesting that excessive production of NO plays an important role in septic

shock (57, 58). Importantly, melatonin has been shown to regulate NO synthesis. Following on from these studies, Maestroni *et al*. investigated whether melatonin could influence the pathology of septic shock (20). Indeed, melatonin-treated mice were protected from LPS-induced shock and reduced mortality correlated with reduced NO synthesis (59). It has been recently reported that melatonin inhibits expression of iNOS in murine macrophages via suppression of NF-κB (60).

To summarize, melatonin is both a positive regulator of immune responses and a negative regulator of inflammation.

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