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Melatonin – a pleiotropic molecule involved in pathophysiological processes following organ transplantation

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Summary

Mammals adjust their physiology in response to seasonal changes to environment (i.e. photoperiod, temperature, food availability). These changes are thought to predominantly occur for the conservation of energy during winter, by pervasive changes such as the inhibition of reproduction. Previous reports have suggested that circannual changes also occur to the immune system. In mammals, this chronological effect may be dependent on photoperiod, and evidence exists to suggest that there is a great deal of immune variation in response to light, or circadian rhythm. This is a clinically relevant, yet under-reported area of human transplantation. The aim of this review is to discuss immune variation, with specific emphasis on melatonin secretion, in the context of organ rejection, infection, neoplasia formation, and immunosuppression.

Keywords: cancer; immunomodulation; infection; melatonin; rejection; transplantation.

Background

Solid organ transplantation is a useful therapeutic tool for the treatment of patients with end-stage organ failure. However, major caveats to transplantation include acute and chronic rejection, both of which are immune-mediated phenomena. Graft rejection occurs via the recognition of donor antigens by the recipient immune system. In an attempt to prevent graft rejection, all organ transplant recipients are treated with continuous and permanent immunosuppression. This in turn increases the risk of infection and neoplasia formation. As a result, the 5-year survival following transplantation is predictably low (i.e. 90% for renal, 75% for heart, 72% for liver, 55% for lung and only 50% for heart–lung). Furthermore, the clinical course (i.e. rejection, infection, malignancy) and response to immunosuppression of transplant patients are notoriously difficult to predict. Such disparity in the clinical outcome of transplant recipients occurs as the result of the vast variations in the genes encoding the immune system, and also in the fundamental mechanisms of the immune response (i.e. redundancy and compensation). However, evidence exists to suggest that the immune system is also affected or regulated by circadian rhythms. Such factors are rarely considered in the transplant setting yet have the potential to significantly alter clinical course in this patient cohort. A probable explanation of immune circadian variation is melatonin secretion, which represents the predominant factor in circadian regulation and has known immunomodulatory mechanisms.

Melatonin

Melatonin (*N*-acetyl-5-methoxytryptamine) is predominantly produced in the pineal gland by pinealocytes. Its secretion is controlled by the suprachiasmatic nucleus in the hypothalamus, which receives light/dark signals from the retina.[1] When 'dark signals' are received, the pineal gland secretes melatonin. This allows melatonin to regulate circadian rhythm, where the duration and concentration of melatonin represent biologic markers of time of day and seasonal clock.[2] However, melatonin is a pleiotropic

compound, affecting multiple systems. Early evidence suggested that products of the pineal gland are involved in immunoregulation, as pinealectomized animals cannot generate complete immune responses following the induction of experimental allergic encephalomyelitis or skin transplantation.[3] The immunomodulatory pineal product was later identified as melatonin, where melatonin inhibition resulted in a depressed antibody response and inferior cytotoxic responses during mixed lymphocyte reactions[4] (collectively reviewed in ref. 5). More recently, melatonin production has also been identified in lymphocytes (reviewed in ref. 6), and is thought to be strongly associated with interleukin-2 (IL-2) secretion.[7] This is an important finding in the transplant setting, as IL-2 is required for T-cell proliferation and clonal expansion, secretion of interferon-γ (IFN-γ), B-cell maturation and differentiation, and natural killer (NK) cell activation, all factors involved in allorecognition and response. Furthermore, the mode of action of the most commonly used immunosuppressant, cyclosporin, is in the inhibition of IL-2 transcription (where cyclosporin binds to an immunophilin termed cyclophilin, which inhibits calcineurin, a molecule essential for the transcription of IL-2). Therefore melatonin has the capacity to alter clinical outcome after organ transplantation (see Figs 1 and 2).

Graft rejection and melatonin

The adaptive response

Graft rejection occurs via recipient alloimmune-dependent processes against donor major histocompatibility complex (MHC) antigens, (or donor MHC-derived peptides) which are presented by dendritic cells (DC), via self MHC molecules to T cells.[8] This process induces allospecific T-cell reactivity and ultimately graft cell loss.[9] Melatonin may play a pivotal role in the regulation of this process. For example, melatonin supplementation in immunosuppressed (cyclophosphamide) animals results in an increase in $CD4^+$ T-cell activity, and also in IL-2 secretion.[10] Furthermore, melatonin supplementation enhances antigen presentation from antigen-presenting cells (APC) to T cells via the specific up-regulation of MHC class II molecules, and further increases tumour necrosis factor-α (TNF-α) and IL-1 secretion.[11] In the early post-operative period following transplantation, donor-derived APC extravasate to the recipient lymph nodes, where they present allopeptides directly to recipient T cells, and recipient-derived APC migrate and diapedese into the donor organ, where allopeptide modification and presentation also occur. This represents the first step in graft rejection, via direct and indirect allorecognition, and interestingly melatonin directly augments these processes. Following antigen presentation to $CD4^+$ T cells, the clonal expansion of allospecific cytotoxic T cells is required. Melatonin augments peripheral blood mononuclear cell (PBMC) proliferation *in vitro*, via the inhibition of production of T helper type 2 (Th2) cytokines, such as IL-10.[12] Furthermore, melatonin increases the production of pro-inflammatory cytokines such as TNF-α, IFN-γ and IL-12 in animal models.[13] Following the clonal expansion of allospecific T cells, mobilization to the transplanted organ occurs, and again melatonin is involved in this process. Melatonin increases chemotaxis of PBMC and neutrophils *in vitro*, and leucocyte chemotactic responses/chemokine expression are increased in humans administered oral melatonin supplementation.[14] Melatonin can further increase chemotaxis via the up-regulation of chemokine production at the sites of inflammation, a process which may be synergistic with TNF-α.[15] Collectively, these studies suggest that melatonin can augment the adaptive processes of allorecognition and alloresponse. However, other immunological components also play a role in graft rejection, including humoral components such as B cells, antibody production/deposition, and innate components such as NK cells and monocytes.

The humoral response

The humoral response plays an important role in solid organ rejection. Antibody-mediated rejection occurs following the production of donor-specific antibodies by B cells. B cells infiltrate donor organs during graft rejection, where they secrete inflammatory cytokines, inducing adaptive Th1 responses. Furthermore, B cells have the capacity to present antigen to recipient T cells, inducing exaggerated adaptive responses. In animal models, melatonin inhibition (via pinealectomy) results in the down-regulation of antibody production (in response to an antigenic challenge).[16] In other studies, animals exposed to short day periods have significantly impaired T-cell responses and immunoglobulin G (IgG) production (following a primary antigen challenge), compared to animals exposed to long day periods.[17] These specific antigen responses are further enhanced following a second antigenic challenge, where IgG concentration remains low in comparison to a significantly elevated IgG concentration in long-day-treated animals. This process suggests that melatonin significantly influences antigen-specific memory and antibody production, two processes closely involved in allograft rejection.

The innate response

Natural killer cells are also directly involved in graft rejection, where they infiltrate organs early[18] and respond via direct cytotoxic responses to donor tissue, and via the stimulation and activation of a range of immune cells (i.e. T cells, B cells, DC, monocytes and endothelium). Several studies have attempted to delineate the effects of

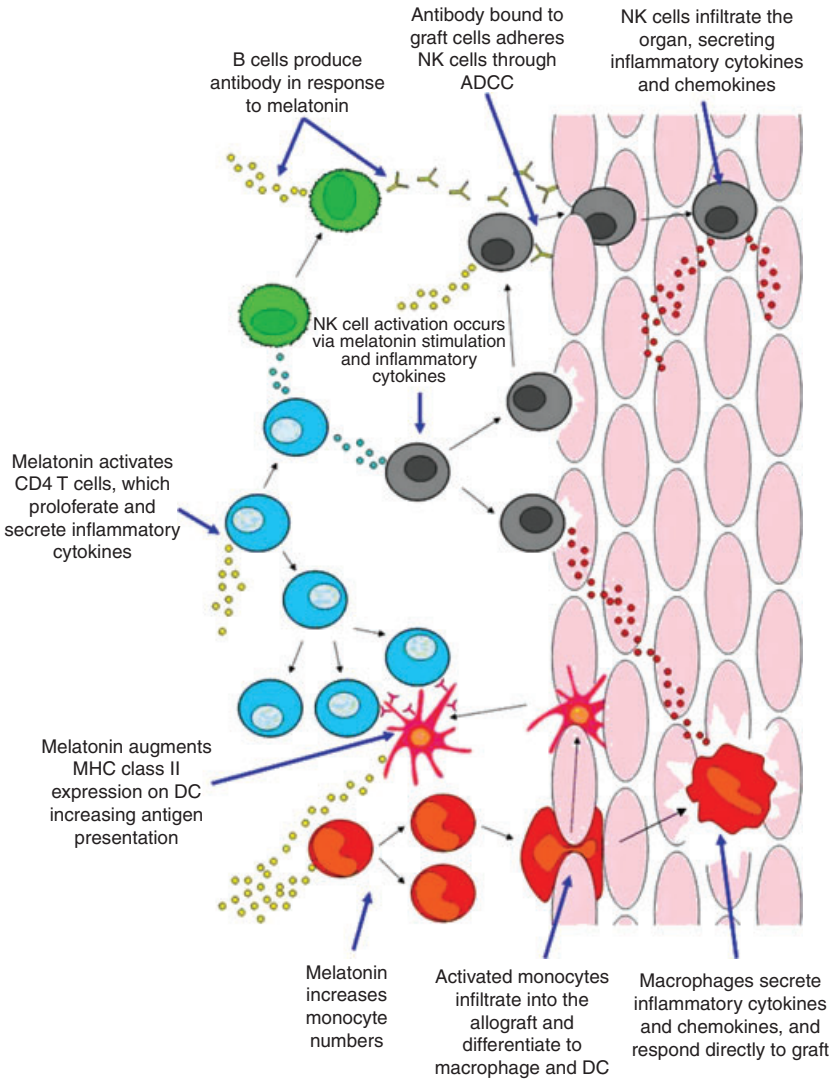

Figure 1. The inflammatory effects of melatonin on graft rejection. Melatonin increases $CD4^+$ T-cell activity, inducing cell proliferation and the synthesis of pro-inflammatory cytokines. Monocyte generation and recruitment occurs, allowing monocyte differentiation to macrophages and dendritic cells (DC). Melatonin augments major histocompatibility complex (MHC) class II expression and induces the production of tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1) and IL-12 by DC, increasing $CD4^+$ T-cell activation. B-cell maturation occurs via direct $CD4^+$ T-cell responses, and melatonin induces the production of antibody. Antibody binds to allograft, inducing direct effects, and also binds Fc $receptor^+$ cells such as natural killer (NK) cells. Melatonin also increases NK cell populations, which infiltrate the allograft, secrete inflammatory cytokines and respond directly.

melatonin on NK cells, with contradictory results. For example, in animal tumour models, melatonin supplementation appeared to have a beneficial effect on survival, but did not alter NK cell numbers.[19]Yet in other animal models, melatonin supplementation has been shown to maintain NK cell progenitor numbers in the bone marrow, and also to increase the number of mature NK cells in the spleen.[20] In the same study, the authors reported that melatonin supplementation was associated with a significant increase in monocyte numbers in both the bone marrow and the spleen. Monocytes have a well-reported role in graft rejection, where they infiltrate the organ rapidly following transplantation, and represent a regenerative source for DC and macrophages. They have the capacity to mediate immune responses because they are capable of secreting both inflammatory and anti-inflammatory (Th1/Th2) cytokines and chemokines according to a given stimulus. Melatonin may represent an inflammatory stimulus to monocytes, as evidence in humans suggests that monocyte function is directed towards an inflammatory phenotype during the night, where production of IL-12 is increased and IL-10 is decreased.[21] In the transplant setting, IL-12 is required for T-cell/granulocyte proliferation and NK cell activation, whereas IL-10 prevents (CD3-dependent) proliferation of T cells, antibody production by B cells, and inflammatory cytokine synthesis.

Animal studies of transplantation

Collectively, experimental and translational studies suggest that melatonin promotes Th1 immunity, through augmentation of cell proliferation, movement and cytokine production. Yet an animal cardiac transplant model provides contradictory evidence suggesting that melatonin inhibits, rather than augments, immune responses to the allograft.[22] In this study, animals were supplemented with low (20 mg/kg) or high (200 mg/kg) doses of melatonin,

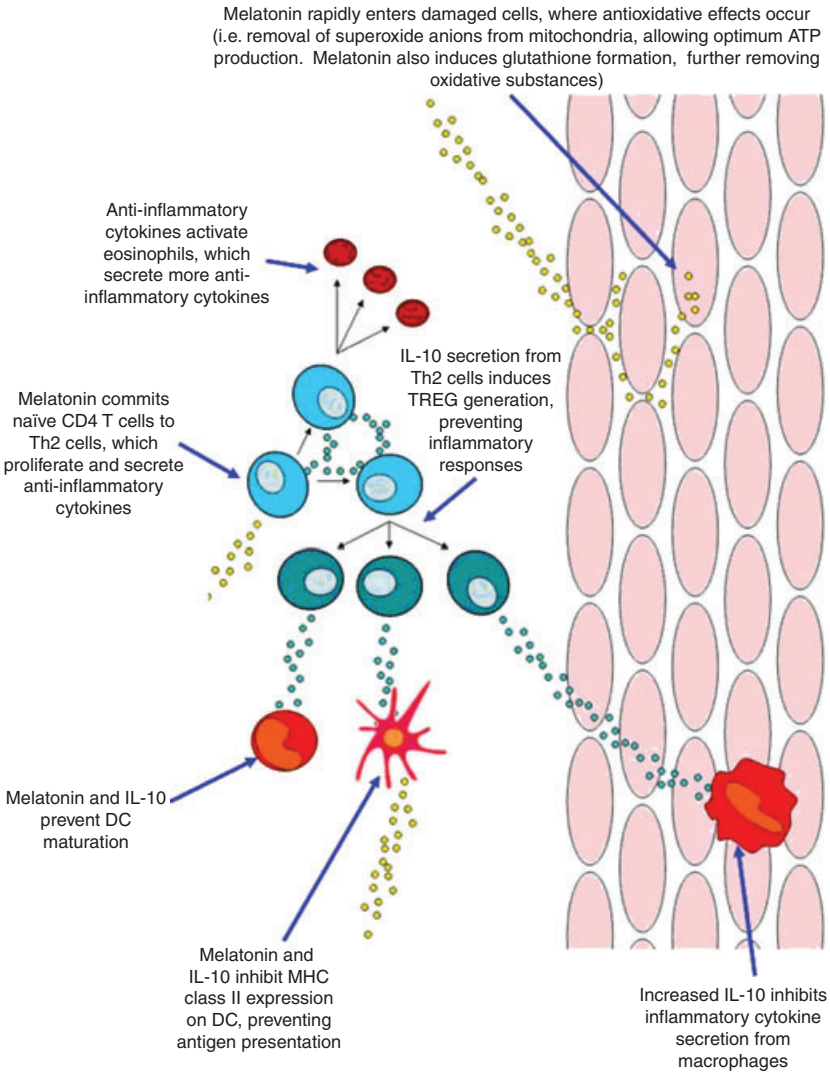

Figure 2. The anti-inflammatory effects of melatonin on graft rejection; melatonin commits naïve $CD4^+$ cells to T helper type 2 (Th2) cells, which then secrete anti-inflammatory cytokines, including interleukin-10 (IL-10). This induces the generation of IL-10-producing TREG cells, which directly prevent inflammatory processes. The IL-10 inhibits the production of inflammatory cytokines from macrophages, and prevents the normal maturation of dendritic cells (DC). Major histocompatibility complex (MHC) class II expression is also down-regulated on DC, preventing antigen presentation. Melatonin directly reduces graft immunogenicity and damage by preventing mitochondrion-dependent apoptosis. This occurs via the removal of superoxide anions generated during ATP production. Melatonin activates other cell-protective molecules, including glutathione, preventing graft cell apoptosis.

and compared with an untreated control group. The study reported a normal circadian rhythm of melatonin (with low trough level reported at 18.00 hr). The concentration of melatonin was similar between the untreated and the low-dose groups, but was significantly elevated in the high-dose group. Following cardiac transplantation, the untreated group experienced rejection at a mean of 6·3 days, the low-dose melatonin group at 7·3 days and the high-dose melatonin group at 12·3 days. This surprising finding indicates that melatonin supplementation abrogates alloimmune responses, preventing rejection. These data imply that melatonin does not selectively commit the immune system to an inflammatory phenotype, but specifically 'regulates' an immune response, which is a direct contradiction to the preponderance of existing research. Other studies suggest that melatonin may further inhibit pro-inflammatory processes. For example, following lipopolysaccharide stimulation, murine macrophages up-regulate nuclear factor-κB (NF-κB) activity. The NF-κB is required in several inflammatory mechanisms including immune cell maturation, activation and proliferation, and the production of nitric oxide (which acts as an immunoactive free radical), via inducible nitric oxide synthase (iNOS) induction.[23,24] Yet co-culture of lipopolysaccharide-stimulated macrophages results in a direct, dose-dependent inhibition of NF-κB production.[25] In the same model, melatonin also dose-dependently inhibited NO production from macrophages. This effect is not unique to macrophages, as NF-κB activation in HeLa S3 cells (via TNF-α stimulation or ionizing radiation) is also inhibited following melatonin co-culture.[26]

Concentration-dependent effects

As a further contradiction to this work, melatonin may have a concentration-dependent effect on the immune system. For example, lymphocyte proliferation is inhibited by high concentrations of melatonin (> 1 nm) following T-cell-specific mitogen stimulation.[27] An inhibitory effect is not observed below this concentration. Indeed, previous

reports demonstrate that biological levels of melatonin are associated with an increase in T-cell proliferation and the up-regulation of pro-inflammatory cytokine synthesis.[10] Other *in vitro* studies have reported similar findings, where increasing concentrations of melatonin induce T-cell proliferation through a dose-dependent mechanism, up to a peak concentration.[28] When melatonin concentrations exceed this value, T-cell proliferation is inhibited.

Anti-oxidative effect

Another potential mechanism by which melatonin may exert beneficial effects following transplantation, is in the inhibition of cellular damage caused by surgical stress, and ischaemia-reperfusion injury (IRI). This has been demonstrated in animal models of hepatic IRI, where melatonin supplementation exerts a protective effect on the liver.[29] Specifically, melatonin reduces neutrophil recruitment, increases the anti-oxidant molecule glutathione, and decreases oxidative substances. Furthermore, the numbers of apoptotic cells are decreased following melatonin supplementation. The anti-oxidative role of melatonin may be of further benefit during graft rejection, by promoting cell repair and removing reactive oxygen species. Melatonin has multiple anti-oxidative functions, including the prevention of calcium overload, removal of toxins such as quinones and pro-oxidative enzymes, prevention of mitochondrial damage, and inhibition of cyclo-oxygenases (reviewed in ref. 30). Therefore, melatonin may reduce graft immunogenicity following transplantation, directly improving clinical outcome. Additionally, other authors have reported associations between melatonin supplementation and an increase in anti-inflammatory cytokines. For example, Raghavendra *et al.* reported that treatment of antigen-primed mice with melatonin results in an increase in IL-10 and a decrease in TNF-α,[31] a phenomenon which would impair inflammatory processes that lead to graft rejection.

Cancer

To add further confusion and controversy to the mechanism of action of melatonin, several authors have reported an inhibition of transformed cell growth, including breast and prostate cancers,[32,33] which may represent a potentially useful immunotherapeutic role in cancer treatment as a Th1 immunostimulant.[34] However, this is a direct contradiction to animal models of cardiac transplantation, where Th1 enhancement results in aggressive allograft rejection. A plausible explanation of the coexistence of a beneficial role for melatonin in preventing graft rejection and cancer may be linked to the pleiotropic nature of the molecule. Melatonin can regulate immune responses, act as an anti-oxidant, and also alter the mitogenic signal transduction pathways required for neoplastic cell proliferation. Many transformed cells metabolize fatty acids to smaller molecules which are required for cell proliferation (an obvious hallmark of a neoplasia). Melatonin can prevent the uptake of fatty acids by transformed cells, so preventing cell proliferation. In animal models, perfusion of tumour cells with melatonin reversibly blocks fatty acid uptake, and prevents cell proliferation, which is independent of an immunoregulatory mechanism.[35] Obviously such a phenomenon is important in the transplant setting, where cancer represents a major cause of morbidity and mortality.

Infection and melatonin

Bacterial and viral infections are an additional cause of morbidity and mortality following transplantation. As well as anti-oxidative and immunostimulatory properties, melatonin also possesses antibacterial and antiviral activity. For example, *in vitro* melatonin supplementation to bacterial cultures (including *Staphylococcus aureus*, and nosocomial bacteria including *Acinetobacter baumannii* and *Pseudomonas aeruginosa*) results in the inhibition of microbial growth, in the absence of immune variables.[36] Treatment of humans showing symptoms of herpes virus infection (a very common viral infection in the transplant population) with melatonin (in combination with magnesium, phosphate and fatty acids) results in major regression of the virus (in 95·7% of the patient cohort $n = 70$).[37] In murine septic shock models, melatonin supplementation has been reported to increase survival by down-regulating pro-inflammatory cytokines and also lipid peroxidation levels in the brain.[38]

Immunosuppression

All transplant recipients require immunosuppression following transplantation, in an attempt to impede the recipient immune response to the donor organ. The most commonly used agent, cyclosporin, is known to deplete melatonin concentrations in animal models.[39] Other immunosuppressive agents, including rapamycin, have similar depletory effects. However, the consequences of this are unclear because the roles of melatonin in the transplant setting are ambiguous.

The melatonin receptors

Melatonin appears to have several, beneficial, yet conflicting modes of action (i.e. preventing immune processes directed towards the graft, while clearing viral infections and preventing cell transformation). A potential reason for this phenomenon is through ligation with unique melatonin receptors. There are two main membrane-bound receptors, termed MT1 (formerly Mel_{1a}) and MT2 (formerly Mel_{1b}). These are G-protein-coupled receptors,

which are widely distributed (i.e. smooth muscle cells, mammary tissue, the gut, liver, kidney, bladder, ovary, testis, prostate, skin, myocardium and immune system),[40] and have proposed individual functions. For example, binding of MT1 in the suprachiasmatic nucleus is thought to be the major control of circadian rhythm. On coronary arteries, MT1 ligation results in vasodilation, whereas MT2 ligation induces vasoconstriction.[41] Other authors have suggested that MT1 alone is responsible for immunomodulation,[27] however, this has yet to be proven and appears unlikely because of the presence of both MT1 and MT2 on immune cells. Melatonin can also bind to orphan nuclear retinoid receptors of the receptor tyrosine kinase-like orphan receptor (ROR) and retinoid Z receptor (RZR) families. In particular, ligation of (ROR)γ-t by melatonin exerts several immunostimulatory effects. For example, RORγ-t ligation on T cells protects against cell death by inhibiting Fas ligand expression.[42] Following primary antigen presentation and T-cell receptor stimulation, RORγ-t expression is up-regulated, increasing the efficacy of melatonin in T-cell survival. B-cell-derived RORγ-t and RZRα ligation with melatonin results in the stable production of several pro-inflammatory cytokines by B cells.

The melatonin receptors are clearly widespread and multifunctional, allowing melatonin to act as a pleiotropic, physiological modulator.

Melatonin supplementation following organ transplantation

Melatonin can be purchased in several countries without prescription, and is widely used as a sleeping aid and a preventative measure for jetlag. In a relatively small meta-analysis (n = 651), short-term melatonin supplementation was considered safe, with no reported adverse events.[43] Melatonin supplementation is also considered as a potential 'immune function enhancer', because of its immunostimulatory properties, and is considered a therapeutically useful supplement in the elderly. However, as discussed, melatonin exerts effects on several physiological processes (i.e. seasonal reproduction, sleep, bone growth, free radical clearance, viral toxicity, T/B-cell priming, T-cell survival, NK cell activation, monocyte proliferation etc). In the transplant setting, it remains unclear whether melatonin supplementation would be advantageous.

Conclusion

Melatonin is a multifaceted molecule with immunostimulatory, anti-oxidative, anti-apoptotic, antibiotic and antiviral properties, which is likely to interact with many pathological and physiological processes observed in transplantation. Despite an inflammatory phenotype, melatonin administration is associated with protection from ischaemia–reperfusion injury and prolonged graft survival following transplantation in animal models. However, other authors have demonstrated that melatonin administration results in an increase in Th1 cytokines and inflammatory immune cells. Therefore the inhibitory effect of commonly used immunosuppressive agents may be of benefit or detriment. This also raises the important question of when to administer immunosuppression. If melatonin 'skews' the immune system to an inflammatory phenotype, should transplant recipients be administered immunosuppression during the night, and not during the day? Clearly this area of research, although greatly understudied, is of clinical significance. Supplementary work is required to provide understanding of melatonin biology following transplantation, and to assess melatonin as a potential therapeutic agent. Additionally, the immunoregulatory potential of melatonin needs to be evaluated in other inflammatory conditions.

Disclosures

The authors have no disclosures to disclose.

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