Review Article

Oxidative Stress and Immunosenescence: Therapeutic Effects of Melatonin

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Age-associated deterioration in the immune system, which is referred to as immunosenescence, contributes to an increased susceptibility to infectious diseases, autoimmunity, and cancer in the elderly. A summary of major changes associated with aging in immune system is described in this paper. In general, immunosenescence is characterized by reduced levels of peripheral naïve T cells derived from thymus and the loss of immature B lineage cells in the bone marrow. As for macrophages and granulocytes, they show functional decline with advancing age as evidenced by their diminished phagocytic activity and impairment of superoxide generation. The indole melatonin is mainly secreted in the pineal gland although it has been also detected in many other tissues. As circulating melatonin decreases with age coinciding with the age-related decline of the immune system, much interest has been focused on melatonin's immunomodulatory effect in recent years. Here, we underlie the antioxidant and immunoenhancing actions displayed by melatonin, thereby providing evidence for the potential application of this indoleamine as a "replacement therapy" to limit or reverse some of the effects of the changes that occur during immunosenescence.

1. Introduction

All organisms experience the inevitable biological process referred to as aging. In general, aging is characterized by a time-dependent functional decline that leads to increased morbidity and mortality as a consequence of the cell's incapacity to face external and internal challenges. Although aging is an extremely complex, a multifactorial process that has been the subject of considerable speculation, accumulated evidence identifies free radicals as a source of damage to cellular structure and function [1].

Among the countless theories proposed for aging, the free radical theory of aging (also known as oxidative stress theory) put forward by Harman in 1956 [2] has received extensive support. This theory proposes that organismal deterioration that occurs as a result of increasing longevity is specially a consequence of the persistent accumulation of free radical-mediated damage to essential molecules, which gradually compromises the function of cells, of tissues, and eventually of the organism itself [3]. Consequently, aging may be viewed as a process of irreversible injuries associated with accumulated oxidative debris.

Since it was posed, the oxidative stress theory of aging has been continuously studied and modified [4, 5], giving a central involvement of mitochondria in determining the timing of senescence, that is, lifespan, as these organelles generate a disproportionately large amount of oxygen-based free radicals and related nonradical species in cells [6]. Nevertheless, despite the fact that the mitochondrial oxidative stress theory of aging is one of the most plausible theories for explaining aging, it has also received some criticisms in the last few years since some groups have proven that knockout mice for antioxidant enzymes did not show any sign of accelerated aging, thus suggesting that mitochondrial oxidative stress may not be causal for age-related degenerative phenomena [7].

Traditionally, oxygen-based free radicals are designated as reactive oxygen species (ROS), whereas nitrogen-based toxic reactants are generally referred to as reactive nitrogen species (RNS). Both ROS and RNS arbitrarily mutilate

macromolecules in the area of where they are produced, this mutilation leading, in many cases, to death of the cell via programmed cell death or apoptosis [8, 9]. Oxidative stress is a condition in which the redox balance between oxidants and antioxidants is disrupted, thereby tilting the equilibrium towards an oxidized state [10]. To counteract the harmful actions of ROS, aerobic cells are equipped with a series of antioxidant enzymes that metabolize toxic reactants to less reactive or totally innocuous molecules. Superoxide dismutases (SODs), glutathione peroxidase (GPx), and catalase are among these antioxidative enzymes. However, this protective machinery seems to be impaired with aging. In particular, SOD activity has been shown to decrease in aged individuals [11–13] although this finding remains disputed [14]. Conversely, catalase and glutathione peroxidase activities have been reported to be augmented with aging, which might reflect a compensatory response to extremely elevated basal levels of ROS/RNS in cells from aged individuals [13].

Furthermore, melatonin is a powerful antioxidant produced naturally by the pineal gland that exhibits relevant antiaging properties [15–18]. Obviously, the use of therapeutic drugs that are intended to improve the quality of life in the elderly implies the identification of molecules that have both antioxidant and immunoenhancing capabilities. In this sense, some of the evidence suggesting that melatonin is efficient to combat age-related deterioration in immune function will be summarized and discussed in this paper with the objective of fostering melatonin as a potential therapeutic agent for enhancing overall quality of life in the elderly.

2. Aging and the Immune System

During aging, the immune system loses functionality and responsiveness. This deterioration is closely linked to a decreased capacity of the immune system to respond to antigenic stimulation and contributes to the increased susceptibility to infectious diseases and cancer in the elderly [19]. This age-associated decline in immune function, which is known as immunosenescence, results in altered cytokine microenvironment and impairment of both innate and adaptive immunity [20].

In general, all immune cells are affected by aging, thereby contributing to the high vulnerability to infections and increased mortality observed in the elderly [21]. Concerning the macrophage, it has been suggested that the existence of a direct relation between age and macrophage activation seems to be responsible for the presence of a subclinical chronic inflammatory process in the elderly. This increase in proinflammatory status at an organismal level, caused by chronic age-related stimulation of the macrophage, is referred to as "inflamm-aging" [22]. Thus, enhanced macrophage ability to produce proinflammatory mediators such as interleukin (IL)-1, IL-6, and IL-8 occurs in both healthy aged subjects and individuals showing pathological aging [22, 23]. Nonetheless, this phenomenon is only a part of the whole spectrum of change characteristic of immunosenescence, and indeed the macrophage is not the only cell

involved in the aging process. The progressive functional T and B lymphocyte deficits have been also suggested as the main responsible factors for age-associated disorders [24]. Certainly, lymphocytes are also largely affected during immunosenescence, and the continuous age-related antigenic stress provokes a variety of changes even in the most evolutionarily recent immune system. These alterations include the expansion of memory B cells, the decrease and even the exhaustion of naïve T cells, and the shrinkage of the T-cell repertoire [25]. Likewise, the reduction both in the number of naïve T cells and in their responsiveness with increased age causes the decline of specific immunization response in aged individuals [26].

As for granulocytes, a functional impairment of these cells has been found in elderly individuals, including diminished intracellular phagocytic capacity, decreased chemotactic activity, degranulation in response to Gram-positive bacteria, and reduced ability to respond to survival factors such as granulocyte macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and lipopolysaccharides (LPS) [27, 28]. In this vein, the attenuation of Fc-mediated phagocytosis in the elderly has been suggested as the major factor for the age-related decline in neutrophil function [27, 28]. Moreover, a reduction in superoxide production of granulocytes has been reported in centenarians, irrespective of subject's health conditions [29].

Furthermore, the activity of natural killer (NK) cells during aging has been extensively studied, and different results have been reported. Strikingly, the most consistent data indicate an increase in cells with high NK activity with advancing age [29, 30]. In fact, cells from healthy centenarians can efficiently kill target cells [31]. This age-associated increase in NK cell number has been interpreted as a compensatory response to overcome the generally decreased immune function that could otherwise trigger neoplastic growth [32]. However, it has been found that aging may severely affect cytokine production of NK cells. Indeed, NK cells of elderly subjects exhibited a diminished production of cytokines in response to IL-2 [33]. Similarly, it has been shown a substantial impairment in the production of mRNA transcripts encoding several cytokines in NK/LAK (lymphokine-activated killer) cells of aged mouse [34].

3. Oxidative Stress and Immunosenescence

Although aging is not considered as a disease by itself, it makes the organism more vulnerable to many of them, including diabetes, obesity, atherosclerosis, cardiovascular diseases, and neurodegenerative diseases [35]. Two essential biochemical mechanisms link immunosenescence to oxidative stress: a reduction in cellular functions owing to oxidative damage of proteins, lipids, and carbohydrates and apoptotic cell death triggered by the accumulation of oxidative debris. The increased amount of free radicals observed in many aged cells has been reported in cells of the immune system as well [36]. In addition, the levels of MnSOD, which is an antioxidant enzyme located in

the mitochondria and protects macrophages from apoptosis induced by oxidized low-density lipoprotein (LDL), are also decreased in aging macrophages [37] thereby contributing to the increased cellular oxidative stress [36, 38].

The observed oxidative modifications occurring on different macromolecules have been shown to compromise the functionality of subcellular organelles, compartments, and membranes [39]. In this sense, the alterations in membrane lipids composition and function due to an increase in the amount of polysaturated and oxidized fatty acids affect activation of T cells and then contribute to human immunosenescence [40, 41]. Moreover, change and damage to the membrane composition also influence receptor-mediated functions of dendritic cells, including the phagocytic clearance of pathogens [42]. Likewise, a consistent decline in the proteolytic activity of the proteasome has been demonstrated with advancing age, implicating an important role for the proteasome in immunosenescence. Besides the inability to clear-damaged proteins, loss in proteasomal proteolysis has far-reaching implications within the immune system. This includes lowered T-cell functional response, reduced antigenic peptide generation for binding to MHC (major histocompatibility complex) class I molecules, decreased maturation of dendritic cells, and, ultimately, dysregulated proliferation because of altered regulation of the cell cycle [43]. Finally, T and B cell plasma membrane receptors, which are directly involved in immune recognition, have also been shown to be affected by oxidative stress. As a matter of fact, it has been reported that many events of T-cell receptor (TCR) signal transduction, such as protein tyrosine kinase (PTK) and mitogen-activated protein kinase (MAPK) activation, are known to be altered with advancing age due to oxidative modification [44]. Additionally, oxidative inactivation of the CD45 protein tyrosine phosphatase was also described to contribute to T-cell dysfunction in the elderly [45].

A second link between oxidative stress and immunosenescence is the induction of cellular apoptosis following the accumulation of oxidized molecular aggregates. Apoptosis is crucially involved in the age-related remodeling of the immune system, which includes thymic involution and alterations in T cells [46, 47]. In this regard, oxidative stress contributes to damage-induced apoptosis by increasing the number of cells undergoing cell death as a result of the accumulation of oxidatively damaged molecules [48], as shown in aged human leukocytes [49, 50]. In fact, we have observed that unstimulated neutrophils and lymphocytes isolated from elderly patients accumulate higher amounts of ROS, present decreased SOD activity, and are less resistant to cell death compared to those cells obtained from young individuals (Table 1). Furthermore, we have determined that aged neutrophils and lymphocytes are more vulnerable to apoptosis triggered by intracellular calcium overload than those cells obtained from young subjects, as ascertained by the activation of different apoptotic hallmarks (Table 2).

Apart from that, the accumulation of proteins modified with advanced glycation end products (AGEs) has been shown to induce T-cell apoptosis in an oxidative stress-associated and caspase-dependent manner with involvement of

TABLE 1: Reactive oxygen species (ROS) levels, superoxide dismutase (SOD) activity and rates of cell death in unstimulated leukocytes from young and elderly individuals.

	Neutrophils		Lymphocytes	
	Young	Aged	Young	Aged
ROS levels	+	+++	+	++
SOD activity	++	+	++	+
Rate of cell death	+	++	+	++

The levels of ROS, SOD activity, and rate of cell death were all classified on a scale of + (indicating lowest level, activity or rate) to +++ (indicating highest intensity level, activity or rate). Data were taken from Espino et al. 2010 [49].

TABLE 2: Activation of different apoptotic events in thapsigargin-treated leukocytes from young and elderly individuals.

	Neutrophils		Lymphocytes	
	Young	Aged	Young	Aged
MMD	+	+++	+	+++
Caspase-9 activity	+	+++	+	+++
Caspase-3 activity	+	++	+	++
PS exposure	+	++	+	++
DNA fragmentation	+	++	+	++

The degree of apoptosis was classified on a scale of + (indicating lowest apoptosis) to +++ (indicating highest apoptosis). MMD: mitochondrial membrane depolarization; PS: phosphatidylserine. Data were taken from Espino et al. 2011 [50].

the mitochondrial pathway [51]. Similarly, studies in cultured macrophages indicated a positive correlation between exposure to oxidized LDL and cell death [37]. Moreover, the stimulation of macrophages with serum glycated proteins, such as pentosidine, a well-characterized AGE found in plasma and tissue of diabetic and uremic subjects, also leads to a loss of cell viability and presumably to cell death [52].

4. Synthesis and Function of Melatonin

Melatonin, or N-acetyl-5-methoxytryptamine, is a widespread physiological mediator. It has been found in most organisms studied from bacteria to humans. The indole melatonin is mainly secreted in the pineal gland of vertebrates, although it is now known to be produced in many other tissues as well [53]. In the pineal gland, melatonin is converted in two steps from the amino acid tryptophan into serotonin (5-hydroxytryptamine), and then acetylated by arylalkylamine N-acetyltransferase (AA-NAT), before finally being converted into melatonin by hydroxyindole-O-methyltransferase (HIOMT), which represents the rate-limiting step in magnitude of melatonin biosynthesis [54]. The pineal gland synthesizes and releases melatonin primarily during the dark phase. Thus, melatonin levels in the circulation exhibit a distinctive circadian rhythm in which the highest blood concentration is observed at night, while baseline levels are measured during the day [55].

It is well known that endogenous melatonin production wanes in the elderly [56] and that the total antioxidative

capacity of serum correlates well with its melatonin levels in humans [57]. In this regard, the participation of melatonin in slowing the deterioration of tissues and organs due to aging has been proposed many times. Thus, it has been shown that the removal of the pineal gland early in life exaggerates molecular damage in terms of lipid peroxidation, accumulation of 8-hydroxy deoxyguanosine in the DNA, and levels of protein carbonyls, as well as reducing membrane fluidity in old animals [58], whereas exogenous administration of melatonin reduces lipid peroxidation [59]. These results, considered in light of the free radical theory of aging, suggest that the age-associated melatonin reduction may be linked to the increase in oxidative damage observed with age [60].

From a physiological perspective, melatonin has been classically related to the physiological adjustment in circadian rhythms and mediating seasonal reproductive events in photoperiodically dependent species. It also alters the function of other endocrine organs and may be involved in sleep regulation in at least diurnally active species [61]. Moreover, melatonin interacts with the cardiovascular system [62] and has been implicated in metabolic control [63]. From a pharmacological view, the phase-advancing effects of melatonin have been frequently exploited [64, 65], with the indoleamine proven to be effective in the treatment of insomnia [66, 67] and efficient in limiting jet lag when travelling across time zones [68].

Finally, melatonin has a particular ability to neutralize free radicals [69] and prevent tissue damage associated with oxidative stress. Thus, it exhibits both direct scavenging actions against free radicals and related products [70–72], as well as indirect antioxidative actions via its ability to stimulate the cellular antioxidant defense system by increasing mRNA levels and activities of several important antioxidant enzymes, including SOD [12], to promote the synthesis of another important intracellular antioxidant, that is, gluthatione [73], to reduce the activity of the prooxidative enzyme nitric oxide synthase [74], and to diminish free radical formation at the mitochondrial level by reducing the leakage of electrons from the electron transport chain [75]. Additionally, different studies have demonstrated its protective role against oxidative damage induced by drugs, toxins, and different diseases [49, 76–78]. This combination of actions makes melatonin an important agent in combating some signs of aging and/or the initiation of age-related diseases.

Apart from that, melatonin has been recently proven to exert antisenescence actions through the activation of SIRT1, a sirtuin that promotes cell survival by inhibiting apoptosis or cellular senescence in mammalian cells. Thus, it has been reported that melatonin increases SIRT1 expression, which reduces inflammatory and apoptotic signaling related to p53, and diminishes vasoconstriction via increasing nitric oxide bioavailability [79]. Likewise, in a murine model of senescence (SAMP8), melatonin protects neurons against frailty by enhancing SIRT1 expression [80], and subsequently decreasing the amount of the acetylated (active) form of p53 [81].

5. Therapeutic Effects of Melatonin on Immune Function

As the age-related decline of the immune system first appears around 60 years of age coinciding with the reduction of plasma melatonin concentration, much attention has been devoted to the possible interaction between melatonin and the immune system in the last decade [32, 82]. In 1986, Maestroni and collaborators first showed that blockade of melatonin synthesis causes the inhibition of cellular and humoral responses in mice [83]. From that point on, a variety of investigations has revealed several modulating actions of melatonin on immune system.

Exogenous administration of melatonin has been proven to stimulate the production of cells mediating the nonspecific immunity, that is, NK cells and macrophage/monocyte lineage cells, in both the bone marrow and the spleen [84–86]. As both these populations constitute the first line of defense against neoplastically transformed and virus-transfected cells, these findings account for melatonin's ability to halt neoplastic growth and to destroy virus-infected cells. Additionally, the action of melatonin on NK cells has been proposed to reflect, at least in part, the fact that NK cells are exquisitely sensitive to cytokines produced by melatonin-stimulated T helper cells, including IL-2, IL-6, IL12, and interferon (IFN)-γ [86], since the immunostimulatory role of melatonin is exerted mainly on both T helper cells and T-lymphocyte precursors [32]. Likewise, monocyte production stimulated by melatonin has been suggested to be driven either directly [87], because cells of this lineage do possess melatonin receptors [88], or indirectly, in response to the triggered cascade of monocyte-sensitive stimulants, such as IL-3, IL-4, IL-6, and GM-CSF, set in place by melatonin activation of T helper cells [84–86].

Furthermore, melatonin administration has been revealed to upregulate the level of gene expression of transforming growth factor (TGF)-β, macrophage-colony stimulating factor (M-CSF), tumor necrosis factor (TNF)-α and stem cell factor (SCF) in peritoneal exudates cells, and the level of gene expression of IL-1β, M-CSF, TNFα, IFN-γ, and SCF in splenocytes [89]. In addition, an inhibitory influence of melatonin on parameters of the immune function has also been demonstrated. Thus, melatonin has been shown to inhibit the production of proinflammatory cytokines, such as IL-8 and TNFα, in neutrophils [90], suggesting that the indolamine may help to reduce acute and chronic inflammation. Melatonin has been also reported to counteract the inhibitory effect of prostaglandin E2 on IL-2 production in human lymphocytes via its MT1 membrane receptor [91]. In this sense, it has been suggested that melatonin may be involved in the regulation of cytokine production by modulating the activity of T cells and monocytes via nuclear orphan receptor (RZR/ROR)-mediated transcriptional control [92, 93].

A correct modulation of apoptosis may be useful for prolonging the lifespan or at least reducing age-related degenerative, inflammatory, and neoplastic diseases whose incidence increases with age. In this sense, melatonin's immunoenhancing effect not only depends on its ability to

improve the production of cytokines, but also on its anti-apoptotic and antioxidant actions. The first data supporting a role for melatonin in drug-induced apoptosis appeared in 1994, when Maestroni and coworkers demonstrated that melatonin-rescued bone marrow cells from toxicity caused either *in vivo* or *in vitro* by anticancer compounds, with this mechanism involving the endogenous production of GM-CSF [85]. Additionally, it has been indicated that orally administered melatonin can substantially boost the survival of newly formed B cells in mouse bone marrow, thereby providing evidence for a role of melatonin as a checkpoint regulator in early B-cell development [94]. Interestingly, Tan and colleagues found extremely high levels of melatonin in bone marrow cells of rats, with melatonin concentrations in the bone marrow being two orders of magnitude higher than in the circulation [95]. Given the high sensitivity of bone marrow cells to oxidative agents, for example, anticancer agents, the presence of high melatonin concentrations in bone marrow cells could be important to preserve their integrity.

The severe loss of thymocytes with age is the main cause of structural thymic atrophy and thymic weight loss. In this respect, it has been indicated that melatonin administration rejuvenates degenerated thymus and redress peripheral immune dysfunctions in aged mice [96]. This reversal of age-related thymic involution by melatonin is attributable to increments in thymic cellularity caused by both the antiapoptotic and the proliferative-enhancing effects of melatonin [97] although other mechanisms involving glucocorticoid receptor cannot be ruled out [98].

The antioxidant ability of melatonin and its metabolites may also account for its antiapoptotic actions on immune cells [99]. In fact, we have demonstrated that melatonin is able to inhibit intracellular calcium overload-induced leukocyte apoptosis by blocking caspase-9 and caspase-3 processing, which is mainly due to the modulation of both the opening of the mitochondrial permeability transition pore and the activation of the proapoptotic protein Bax [77]. Moreover, we have also proved that the beneficial effects resulting from melatonin administration on leukocyte apoptosis likely depend on melatonin's antioxidant properties, since this protection is unaffected by the MT1/MT2 antagonist luzindole, that is, independent of plasma membrane MT1/MT2 receptor stimulation [78]. More interestingly, melatonin is able to delay damage-induced apoptosis in aged neutrophils and lymphocytes and therefore may counteract, at the cellular level, age-related degenerative phenomena linked to oxidative stress [50]. This fact is especially remarkable as neutrophils from aged individuals show a diminished rescue capacity when challenged with proinflammatory stimuli, such as GM-CSF, GCSF, LPS, or IL-2 [100, 101].
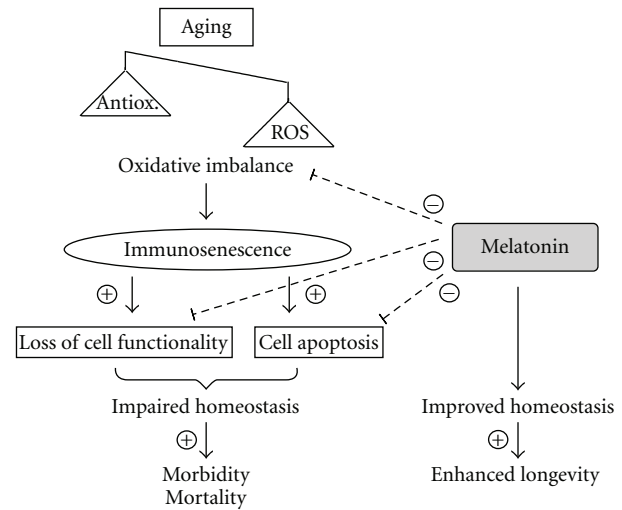

Figure 1: Schematic diagram depicting beneficial effects of melatonin on age-associated deterioration of immune system.

macrophages. In general, these age-associated changes in the immune system render organisms more sensitive to infections, autoimmune diseases, and even to cancer (Figure 1). In the last few years, growing evidence has indicated a tight, cause-effect link between oxidative stress and immunosenescence. Strikingly, several studies have highlighted the reversibility of some of the changes induced by oxidative stress with advancing age, which is particularly important for most cells in the immune system which, having a very short lifespan, are constantly replaced by newly produced elements. Therefore, these cells can potentially benefit from short-term therapies aimed at decreasing oxidative stress. In this sense, it is worth noting the possibility that melatonin supplementation could prevent or delay the functional deterioration of the immune system that accompanies aging and, perhaps, return it to that of the "younger" situation (Figure 1). Indeed, dietary supplementation of melatonin has been shown to ameliorate the attenuated immune responses associated with senescence [102]. Likewise, melatonin-enriched foodstuffs has been proven to modulate serum inflammatory markers in both rats and ringdoves, causing a reduction in proinflammatory markers along with an increase in anti-inflammatory markers which suggests amelioration or reorganization of immunity to a noninflamed state [103, 104]. Based on the experimental data that have accumulated and considering its lack of toxicity, its high lipophilicity, and its large capacity to forestall cell damage, melatonin is one of the most appealing agents to be examined in relation to age-associated deterioration in the immune system and should be considered as a potential agent to improve the quality of life in a rapidly aging population.

6. Concluding Remarks

The age-associated decline in immune function, known as immunosenescence, is characterized by a decrease in the functional activity of NK cells, granulocytes, and

Acknowledgments

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