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Immune Mechanism, Aging, Season and Diseases: Modulatory Role of Melatonin

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Abstract: Immune mechanism of the body plays an important role in arresting neoplastic growth and in controlling infectious diseases. The innate immunity, adaptive immunity comprising of cellular and humoral immunity have distinct roles in fighting against cancer and infectious diseases. The role of neutrophils, monocytes-macrophages, T helper (Th)-1 and Th-2 lymphocytes, B-lymphocytes and cytokines in arresting neoplastic growth and in combating infections and the complex interrelationship among themselves and with neuro-endocrine network in the body has gained much impetus with the discovery of number of receptors and binding surfaces on these cells. The increased incidence of neoplastic and infectious diseases seen in the elderly is attributed primarily to decreased immune function of the body, and termed as immunosenescence. Alteration in circadian rhythmicity of various subsets of lymphocyte population has been documented in the elderly. Similarly recent studies on cancer patients reveal that there exists two distinct types of lymphocytes with some cells exhibiting acrophase during morning and others in the night and circadian variation of lymphocyte population in cancer patients suggest impaired integration of nervous, endocrine and immune responses in neoplastic disease. Seasonal outbreak of some infectious diseases seen in some parts of the world has supported the photoperiodic regulation of immune function with enhancement during short photoperiods and inhibition during long photoperiods. Although the evidences for this are largely derived from animal studies, its application to human studies is still in a preliminary stage. However, the neurohormone melatonin which was shown to have an immunomodulatory role may stimulate immune mechanisms and in this way, melatonin could be a very useful resource for inhibiting neoplastic growth. Melatonin stimulates natural killer cells which are known to attack and destroy cancerous cells by their immunosurveillance mechanism. In addition, Th-1 cells, B-lymphocytes, release of cytokines from immunoregulatory cells are influenced by melatonin. The synthesis of melatonin by lymphocytes and thymus supports an immunomodulatory role for melatonin and its application in the control of infectious and neoplastic diseases.

Keywords: Adaptive immunity, aging, cancer, cytokines, immune mechanism, infectious diseases, innate immunity, lymphocytes, melatonin, season.

INTRODUCTION

Epidemiological studies reveal that the level of immunity of an individual determines his longevity [1]. The existence of "immunological risk phenotypes" that predicts the life span of individuals has also been identified [2]. There is a steady decline in immune function which increases with aging, that has been termed "immunosenescence" and that predisposes an individual to increased susceptibility to infectious diseases, neurodegenerative or proliferative diseases or combination of any of these disease states [3]. Various screening approaches have been recommended for study the decline in immune function in relation to aging and of this SENIEUR protocol provides standard set of guidelines [4]. Immuno-gerontological studies reveal inconsistencies in age-related changes both in specific and non-specific immune functions [5, 6]. These inconsistencies in immunosenescence are reflected at all levels. Influences of nutritional status of individuals [6], as well as the interrelationship between endocrine and immune system [7] are considered primary determinants of immune function and variability of immunosenescence seen in different individuals of the same chronological age [8].The age- associated decline in the levels of various hormones like growth hormone (GH), estrogens, dehydroepiandrosterone and the pineal hormone melatonin have all been suggested as contributory factors for immunosenescence [7]. Among these hormones, melatonin's involvement in modulation of immune responses is of paramount importance [9]. The individual's susceptibility to infectious or proliferative diseases depends upon his own immmunocompetent status, and variations in the degree of susceptibility to infectious or proliferative diseases among individuals of the same chronological age is attributed to degree of variations in immunocompetency and is deter-

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mined by the interplay of various factors like the environmental, genetic, and nutritional status, and the hormonal levels. The immuno-neuroendocrine axis also affects the susceptibility of an individual to various diseases [10] and the importance of ***psychoneuroimmunology*** is gaining much recognition in understanding the competency of immune mechanism among individuals of the same age [11]. In this paper, we will discuss the immuno-modulatory role of melatonin in relation to aging, infectious, and proliferative diseases, namely cancer, with the aim of understanding its therapeutic significance for treating these disease states.

Immune mechanism, Circadian Rhythmicity and Aging

The interrelationship between immune system and the endocrine system is of crucial importance in mediating age-associated degenerative diseases. Aging results changes in humoral immunity as reflected by decrease in the number of B lymphocytes and increase in the levels of immunoglobulin (Ig)A and IgG [12]. Reduction in CD27+ memory cells, CD5+ B cells with low levels of T cells occurs with aging [13]. A decline in organ specific auto antibodies together with an increase of non-organ specific antibodies has been reported in the elderly [14]. Thus the reduced humoral responsiveness and altered antibody mediated defense mechanisms seen in the elderly is caused by the intrinsic primary cell deficit. Moreover the involution of thymus with age results in alteration of gene expression resulting in immunosenescence at cellular, molecular and genetic levels [3].Severe loss of thymocytes with thymic atrophy and thymic weight loss occurs with aging [15]. The age related changes is seen not only in immune mechanisms but also is reflected in nervous and endocrine mechanisms as well since these systems interact with immune system. Bidirectional connections among the immune systems with nervous and endocrine systems mediated by chemo/cytokines are essential for maintenance of body homeostasis [16, 17]. Correlative studies with various lymphocyte subpopulations and serum hormonal levels reveal that a decrease in peripheral B-cell compartment and an increase in activated T-cell compartment were found associated with a decrease of thyroid stimulating hormone (TSH) secretion [16]. From this study the authors suggested that the hypothalamus-pituitary axis play an immunomodulatory role and influence cellular immune responses by releasing various hormones and neuropeptides [16, 17]. An interesting observation made from the above studies was that there exist two distinct immune cellular compartments, the diurnal compartment (lymphocyte subsets with acrophase during the day; CD8, CD16, T cell receptor [TCR]-γδ bearing cells) and the nocturnal compartment (lymphocytes subsets with acrophase during night; CD4, CD20, CD25, HLA-DR). The time-qualified changes in the levels of activities of these lymphocytes show distinct variations in the young and middle age persons as compared to the elderly and are most likely the physiological mechanism for triggering and regulating immune responses [18]. Severe alterations of circadian rhythmicity of variation of TCR-γδ bearing cells have been found in the elderly subjects [17]. As these cells represent the true immune surveillance cells, their altered circadian rhythmicity is likely to contribute to the increased incidence of cancer seen in old-age people [19]. Circadian variations in the levels of other lymphocytes have been documented. In the elderly subjects, the circadian rhythm of CD25 is phase advanced, and the circadian rhythm of total T cells is phase-delayed. The T suppressor/cytotoxic lymphocytes, natural killer (NK) cells and the levels of TCRδ1 are higher in the late morning and show a clear circadian rhythmicity. There is a speculation that TCRδ1 cells are specialized for myobacterial immunity or destruction of 'stressed' autologous cells (acute leukaemia cells) [20]. Increased T cell activity that was seen in this study was interpreted to be the cause for increased frequency of autoimmune response seen in the elderly [21].The alterations of these lymphocytes do not just reflect the patterns of circadian rhythmicity but severely impair the operation of immune system in aging [17]. Study of correlation of lymphocyte subpopulations with hormonal changes in the blood reveals that melatonin and cortisol levels remained normal in all three categories of subjects (young, middle and elderly) studied. However melatonin levels were decreased in elderly subjects of above 80 years [21].

Immune Mechanism and Cancer

Alterations in cytokine microenvironment and decreased functional activities of both innate and adaptive immunity, particularly deficits in both T cell and B cell activities occur with aging [22]. NK cells are the main components of the innate immune system, being responsible for inhibiting cancer and metastasis growth. Some studies [23, 24] have demonstrated the decrease in functional ability of human NK cells with age. NK cells of aged individuals exhibit decreased production of interferon (IFN)γ and chemokines in response to interleukin-2 and interleukin (IL)-6 [24]. However, other studies have shown that the ability of human NK cells to synthesize chemocytokines in response to IL-6 stimulation is preserved in normal healthy individuals of over 90 years [25]. The preservation of NK cell cytotoxic capacity in peripheral blood of centenarians has also been demonstrated [26]. Studies on polymorphonuclear leukocytes of elderly individuals show decreased intracellular chemotactic and phagocytic activity [25, 27]. Functional alterations in the capacity of NK cell activity as well as granulocytic activity with decreased anion superoxide (O_2^-) production have been demonstrated in the granulocytes of aged centenarians [28]. Decreased production of O_2^- in granulocytes is attributed to a reduction of the signal transduction mechanism [29]. The attenuation of Fc-mediated O_2^- generation and phagocytosis seen in the elderly is a major factor for the decline of neutrophil function of elderly individuals [27, 30]. With regard to monocyte functioning, decreased generation of O_2^- and diminished levels of IL-2 have been reported in the elderly [31]. In addition, increased production of pro inflammatory mediators like IL-1, IL-6, and IL-8 has been reported in individuals with pathological aging [32]. An increase of IL-6, the "cytokine of gerontologists" [33], has been reported even in healthy elderly individuals of more than 85 years [34-36]. This increase of IL-6 seen in elderly individuals is one of the major contributory factors for age-associated diseases and increased mortality [37, 38]. Cytometric phenotypic analytical studies reveal that the ability of T cells to promote B-cell activation and antibody production is much compromised in elderly individuals [39]. In addition, decreases of both T-lymphocytes and B-

lymphocytes have been reported in the elderly [40]. Reduction of CD27+ memory B cells, with low T cell numbers also occurs in aged individuals [41]. Significant decrease of cellular immunity, as reflected by decrease in the number of CD3+, CD4+, CD8+ cells, naive T lymphocytes CD4+, CD45RA occurs in the elderly [42]. T-cell alteration in aging is associated with a decrease in the number of naive cells, causing decline in specific immunization response in old age group [43]. Large increase of dysfunctional cytomegalovirus specific CD8+T cells is common in the elderly [44]. Longitudinal studies undertaken in a Swedish population (OCTO studies) suggest that a pattern of immune parameters with low CD4+ cells, increase of CD 8+ cells, and low IL-2 production are predictive of increased mortality rates. [45-47].

Aging, Immune Mechanism and Cancer

Understanding the function of immune mechanisms in aging may provide insights into the complex relationship between immunity and cancer. Studies on knockout mice reveal the role of immune system in controlling the spontaneous generation of tumors. Among aged knockout mice (IFN-$\gamma^{-/-}$ perforin mice), it was found that 50% of them develops lymphomas, lung adenocarcinoma, or sarcoma [48], showing thereby the importance of healthy functioning of the immune system in preventing the development of tumors. NK cells of the innate immune system play an important role in inhibiting cancerous growth and metastatic tumours. This has been revealed by a prospective study undertaken on 3500 middle aged and elderly Japanese over 11 years wherein it was noted that the incidence of cancer was higher in individuals who had lower NK cytotoxic activity [49]. Similar findings of lower levels of NK cells in patients with gastric carcinoma correlated with increased tumor size, metastases and worse prognosis were reported [50]. Unlike the other cells of the immune system, the number of NK cells is actually increased in healthy aged individuals when compared to young or middle aged persons [31, 32]. The increase of NK cells or NK cell receptors on T cells is suggested to be of beneficial in curbing the growth of neoplastic cells through its immune surveillance mechanism [51]. The importance of immune surveillance mechanisms in arresting neoplastic growth has been demonstrated in an experimental study in which crossing of tumor suppressor heterozygous $p53^{+/-}$ onto a $perforin^{-/-}$ mice caused reduction in the age of onset of lymphomas with increased frequency of lymphomas suggesting thereby that the tumor suppressor deficits associated with increase in age is exaggerated by the absence of immune surveillance mechanism [52]. The importance of perforins in suppressing spontaneous lymphomas is also indicated by this study [52]. It has recently been reported that NK cells preferentially target stem cells. Increased NK cell function was seen when these cells were cultured with primary oral squamous carcinoma stem cells (OSCSCs) when compared to more differentiated OSCSCs. Inhibition of differentiation or reversion of cells to the less differentiated phenotypes by blocking NFκB or targeted knockdown of cyclooxygenase-2 **(**COX2) significantly augmented NK cell function. NK cells also cause lysis of human embryonic stem cells (hESCs), human mesenchymal stem cells (hMSCs) and dental pulp stem cells (DPSCs), than their differentiated counterparts. NK cell mediated lysis of these cells, namely OSCSCs, hMSCs, DPSCs were prevented by total population of monocytes, showing thereby that the cytotoxic function of immune effectors is largely suppressed by tumor micro- environment by a number of distinct effectors and secretors. Hence, it is advised that the patients with cancer may benefit from repeated allogenic NK cell transplantation at the site of tumor for specific elimination of cancer stem cells [53]. A newly synthesized indoleamine derivative Ey-6 kills Mc38 tumor cells in a dose-dependent manner (25, 50, 100 μM) with carleticulin induction. An Ey-6 treatment altered the tumor cell microenvironment favourable to anti-tumor immune responses by inducing the secretion of IFNγ from M38 cells, which is considered an important mechanism for inducing antitumor responses. Ey-6 is a novel chemotherapeutic agent that can manipulate the host immunity favourable to eliminate tumor cells by attacking only tumor cells and not attacking normal antigen presenting cell, dendritic cell maturation [54]. Recent development of immunological newer tools has made possible the attack on cancer cells with specificity of immune system. Immunotherapy has been instituted against melanoma, prostate cancer, colorectal cancer, and haematological malignancies [55]. In the tumor microenvironment, there is always a delicate balance between antitumor immunity and tumor oriented pro-inflammatory activity, and modulation of immune cell microenvironment and inflammatory processes represent a novel method of target for therapeutic intervention in the control of malignant diseases. Recently, it was reported that a tumor-derived chemokine ligand (CCL), namely CCL-5, is highly expressed in cancer and plays a critical role in immune escape of colorectal cancer. High levels of CCL-5 expression in human and murine colon cancer cells are associated with high apoptosis of CD8+ T cells and infiltration of T_{reg} cells. RNA interference mediated knockdown of CCL-5 delayed tumor growth in imunocompetent syngeneic hosts, but had no effect on tumor growth in immunodeficient hosts. Hence, controlling the expression of CCL-5 will help in increasing CD8+ T cells and for lysis of tumor cells [56]. As cancer medical therapy is currently based on the use of high doses of cyclophosphamide or anthracyclines which not only kill tumor cells, but also inhibits T cell function or macrophages, the use of immunotherapy for arresting specifically the neoplastic growth without affecting normal bone marrow cells, or antigen presenting cells has become crucial for effective management and control of cancer **[**55].

Circadian Rhythmicity of Neuroendocrine-Immune Mechanisms in Neoplastic Disease

It is suggested that subversion of chronorhythmicity and bodily temporal disarray might result in the development and progression of neoplastic disease. Evaluation of CD3+, CD8+, CD8+ dim, CD20+ lymphocytes, GH, TSH and cortisol shows loss of normal circadian rhythmicity in cancer patients [57]. Loss of circadian rhythmicity of cortisol secretion with increased serum cortisol levels were noted in these patients suffering from lung cancer [57]. The circadian variation in lymphocytes subpopulation seen in lung cancer patients seen in this study is attributed to altered profile of cortsol secretion [57]. Not only cortisol but levels of various other hormones like thyrotropin releasing hormone (TRH), TSH and GH are found to be altered with loss of circadian

rhythmicity in lung cancer patients [58]. One of the main causes of cancer is chronodisruption and internal desynchronization that leads to functional disturbances resulting in impairment of bodily repair and defence mechanisms [59]. Lung cancer patients exhibited clear circadian variations of lymphocyte populations. Mean diurnal levels of CD8 (T suppressor/cytotoxic subset) and CD 8 bright levels were lower and CD 16 levels were higher in cancer patients [59]. The multifrequency structure that characterizes the function of the immune system and the complexity of circadian variations of its different components account for maintenance of normal homeostasis and disruptions of this organization leads to development of neoplastic diseases [58].

Aging, Immune Mechanisms and Infections

Human beings are constantly exposed to a variety of bacterial and viral infections but are endowed with intrinsic mechanisms to protect themselves against these infections. The co-ordinated actions of innate and adaptive immune mechanisms play a major role in this aspect. Immunosenescence is associated with greater incidence of infection, as there is a decline in the ability to mount primary immune response against pathogenic antigens [40]. There is a progressive decline in the function of both innate and adaptive immune system [30, 60]. Age-related deficiencies of adaptive immune system include atrophy of thymus, restriction in the production of naive T cells, and replicative senescence of peripheral T cell pool [30]. These changes of the adaptive immunity contribute to increased susceptibility of the elderly to novel viral infections such as influenza [61]. Infectious diseases like influenza and pneumonia are major health problems in older people and are leading causes of death in the elderly [12]. Class1-restricted CD8+ cells are essential for fighting against infectious agents, form part of the "immunological clock", and reduction in their numbers predict lack of protection against pathogenic microorganisms. Age-related thymic involution with decreased output of new T cells predisposes elderly individuals to a variety of new infectious diseases like pneumonia, urinary tract, skin, and soft tissue infections, and infections from viral origin [12]. The antibodies IgG and IgA generally confer greater protection against bacterial and viral infections and increased levels of these antibodies have been reported in healthy old centenarians [39]. Among the subclasses of IgG1, IgG2, and IgG3 are increased whereas IgM did not increase. IgG1 and IgG3 are involved in the humoral responses to viral infections, whereas IgM are involved with antigens present on the bacteria surface [24]. In addition to age related reductions in the level of auto antibodies and B cells, the ability of T-cells to promote B-cell activation and antibody production also greatly decreased in elderly people [39]. Besides these changes in humoral immune mechanisms of the elderly, innate immune mechanism is very much impaired in the aged people. Both macrophages and granulocytes offer primary resistance to infectious diseases caused by bacterial and viral infections. Regarding granulocytes, accumulated evidence suggests decreased efficiency in the functional activities of these cells, like phagocytosis, chemotaxis, O_2^- production, which are all decreased in the elderly as compared with younger subjects [24]. The ability of mononuclear cells to produce proinflammatory cytokines such as IL-1, IL-6, IL-8, and tumor necrosis factor α (TNFα) is also very much increased in the elderly [61]. Plasma IL-6 levels increase from 50-60 years and continue to increase till the person reaches the extreme age limit [61]. Although the increase in IL-6 levels is suggested as the most powerful predictor of morbidity and mortality in the elderly, it is also found in healthy centenarians and according to Ginaldi *et al.* (2001) [12], "the increase in IL-6 with increase in age is the consequence of the successful adaptation to several stress factors, including infections which occur throughout life" [22]. The fact the immunosenecence is responsible for increased prevalence and severity of infectious diseases has been supported by several studies [62-64]. Recently, in a study conducted in 17 centenarians (the age group from 100 to 105) revealed that IL-22 levels increased in all these healthy centenarians. IL-22 is a proinflammatory cytokine produced by activated T lymphocytes and NK cells. It belongs to proinflammatory cytokine family of IL-10 and stimulates the production of acute phase reactants and thereby promotes antimicrobial defence [65]. Another interleukin that has recently been advocated for fighting against microbial agents is IL-15. IL-15 has been shown to play a major role in the development of inflammatory and protective immune responses to microbial invaders and parasites by modulating immune cells of both the innate and adaptive system [66]. Search for administration of suitable substances that can promote both innate and adaptive immunity in the elderly for improving the quality of life has been on for a number of years, and recent studies have pointed out the use of dietary phenols. Numerous studies have reported curative properties of dietary polyphenols like curcumin, genistein, resveratol and epigallocatechin in cancer [67]. Micronutrients like zinc, selenium, vitamin E have immunoenhancing properties and it is well known that zinc deficiency has been associated with increased bacterial infections and has been used for human immunodeficiency virus (HIV) infected individuals [68]. Use of trace elements and vitamins has been advocated for immunoenhancement of the elderly to fight against infections and cancer [69]. The decline in the production of different hormones like GH, estrogens, dehydroepiandrosterone, and melatonin has been suggested as possible contributory factors of immunosenescence [7]. Hence, hormone replacement therapy for enhancing immune mechanism of the elderly has been advocated [12]. In this context, melatonin, a natural anti-oxidant with immune enhancing properties, has been suggested as a possible therapeutic agent for use in elderly to fight against infections and cancer [8].

Melatonin, its Biosynthesis and Metabolism

Melatonin is synthesized mainly in the pineal gland of all animals and is formed from the amino acid tryptophan, which is converted into serotonin. Serotonin is then acetylated to form N-acetyl serotonin by the enzyme arylakylamine-N-acetyltransferase (AAAT). N-acetyl serotonin is converted into melatonin by the acetyl serotonin methyl transferase (ASMT). A schematic diagram on melatonin biosynthesis is presented in Fig. (**1**). Melatonin is also synthesized by many other organs and tissues in the body like skin, gastrointestinal tract, eye, etc. Notably, melatonin is also synthesized in tissues and organs concerned with immune mechanisms. The findings that melatonin is synthesized in

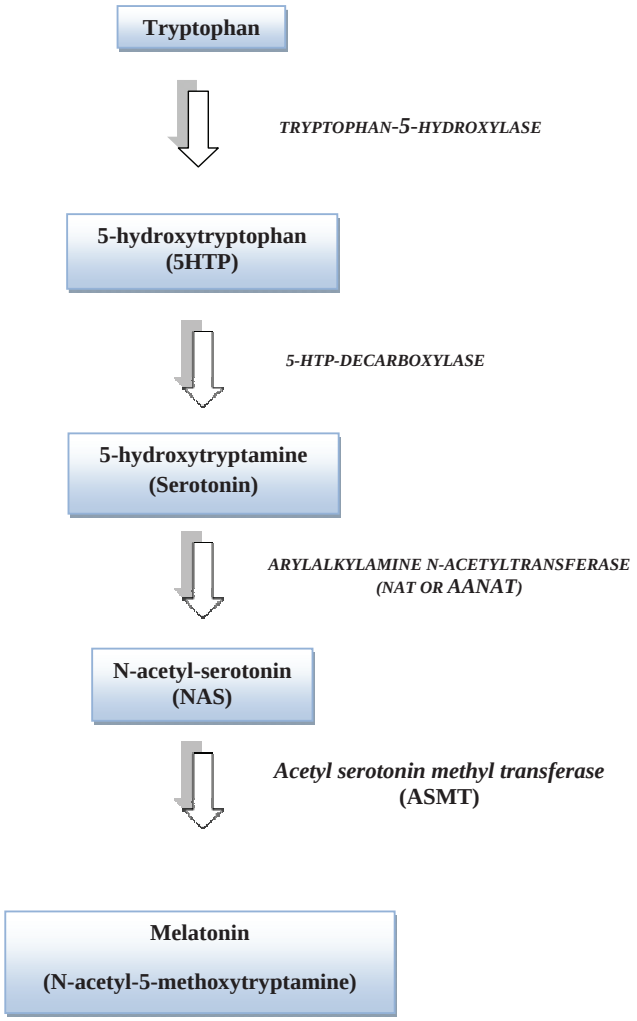

Fig. (1). Melatonin biosynthesis.

lymphocytes [70], and in thymus [71], strongly support that this hormone plays a crucial role in influencing the immune mechanism. In humans and in most of the animals, pineal melatonin synthesis and secretion exhibit a circadian rhythm with low levels in the pineal gland, plasma and other body fluids during day time and high levels during night time [72]. Most of the investigators agree that melatonin synthesis and secretion decrease with age [73-77]. Although melatonin is synthesized by a number of organs in the body, circulating melatonin is derived exclusively from the pineal gland [78]. Being a highly lipophilic molecule, melatonin reaches all tissues in the body within a short period of time. It is released into the cerebrospinal fluid (CSF) and its concentrations in the CSF is relatively high [79]. Circulating melatonin is metabolized mainly in the liver where it is first hydroxylated by cytochrome P450 mono oxygenases and then conjugated with sulphate to form 6-sulfatoxy melatonin. In the brain, melatonin is metabolized by oxidative pyrrole ring cleavage to form kynuramine derivatives. The primary cleavage product is N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) [80]. Its formation by myeloperoxidase is considered to be important in quantitative terms [81]. Melatonin diffuses through biological membranes with ease since it is both water and lipid soluble, and exerts its actions on all cells and tissues in the body. Some of its actions are receptor-independent (such as anti-oxidant actions), while most of its other actions are mediated through membrane, cytosolic or nuclear receptors.

Melatonin Receptors and Signal Transduction Pathways

Melatonin receptors have been detected in numerous tissues and peripheral organs, like gastrointestinal tract, liver, lung, skin, endocrine glands, gonads, kidney, heart, blood vessels, adipose tissue, mammary tissue, lymphoid organs, and many parts of the central nervous system, and the distribution of these receptors has been reviewed [82]. The two membrane melatonin receptors that have been cloned are MT1 and MT2 melatonin receptors which have seven membrane domains and belong to the super family of G-protein coupled receptors [83-85]. Melatonin receptor activation induces a variety of responses that are mediated both by pertusis sensitive and pertusis insensitive G proteins [85]. In the cytoplasm, melatonin may interact with calmodulin [86]. A third melatonin binding receptor known as MT3 has been identified, but subsequently characterized as the enzyme quinone reductase [87]. Melatonin exerts its genomic actions by binding to nuclear receptors of the retinoic acid receptor family retinoic acid receptor-related orphan receptors (ROR)α 1, RORα 2, and RORβ [88, 89]. RORα1 and RORα 2 seem to be involved in immune modulation, as these receptors and their signalling pathways have been identified on immunocytes [90]. Melatonin acts specifically on MT2 receptors expressed in immuno-competent cells and regulates both cellular and humoral immunity [91].

Melatonin's Immunomodulatory Role

The role of melatonin in immunomodulation was explored by one of the authors of this paper (George Maestroni), who was the first in demonstrating that inhibition of melatonin synthesis resulted in inhibition of both cellular and humoral immunity [92]. Decrease in cellularity of thymus and spleen with depressed autologous mixed lymphocyte reaction was noted in mice kept in constant light or injected with β-adrenergic blockers, procedures which block melatonin synthesis. These mice were also unable to mount primary antibody responses to sheep erythrocytes [92]. Late afternoon injection of melatonin increased both the primary and secondary responses to sheep red blood cells [93]. Maestroni *et al*. (1987) postulated that stimulating effects of melatonin on immune mechanisms is both direct as well as through opiodergic pathways [94]. Melatonin's effect in correcting the immunodeficiency status induced by propranolol (β-adrenergic blockers) was abolished by injection of the opiod antagonist naltrexone, showing thereby the involvement of the opiodergic pathways in the mediation of melatonin's immune modulatory functions [94].

Pinealectomy abolished both cellular and humoral responses in white turkey poults (birds), whereas melatonin replacement therapy restored both cellular and humoral responses in these birds, showing thereby the importance of melatonin for the functioning of the immune system [95]. Melatonin's immunoenhancing action is attributed to its direct effects on immunocompetent cells like lymphocytes, NK cells, and granulocyte-macrophage cells. The existence of

specific melatonin binding sites in lymphoid cells provides an anatomical basis for the direct effects of melatonin on the regulation of lymphoid system. By using I^{125}-melatonin as a ligand, high affinity melatonin binding sites and their signal transduction pathways were identified in human lymphocytes [96, 97]. The inhibitory effects of prostaglandin E2 on IL-2 production in human lymphocytes was blocked by melatonin through its actions on MT1 melatonin receptors [98]. Melatonin also increases the production of monocytes and macrophages. The enhanced monocyte production induced by melatonin is suggested to be due to its direct action on melatonin receptors on progenitor cell [90], to the increased sensitivity of monocytes to stimulants such as IL-3, IL-4, IL-6 or to granulocyte-macrophage colony stimulating factor [99, 100]. The stimulatory effect of melatonin on monocyte/macrophages production in rodents has been confirmed by other investigators [101]. Melatonin is suggested to regulate hemopoietic cell proliferation by acting on bone marrow stromal cells which contain receptors for κ-opioid cytokine peptides. By activating these receptors and releasing opioid peptides from these stromal cells, melatonin may regulate hemopoietic cell proliferation [102]. In addition to increasing lymphocytes, monocyte series, melatonin administration increases circulating number of NK cells and also the spontaneous NK cell activity [103]. Melatonin induced increase of NK cell number is due the increased production of cytokines like IL-2, IL-6, and IFNγ from T cells [104].

Melatonin's Action on Thymus and T Lymphocytes

The thymus, as a primary lymphoid organ, has profound effects on the immune system and is often referred as the "organ of youth in mammals". The process of thymic involution is considered as one of the remarkable feature occurring with age [105]. In the earliest study conducted on pineal influence on thymus growth, it was noticed that pinealectomy in young mice caused accelerated involution of thymus [106]. The loss of thymocytes with age is the main cause for structural thymic atrophy and thymic weight loss. The reversal of age-associated thymic involution by melatonin was studied. The thymic cell number of 2 months old mice was $12.6x10^7$ and at 24 month's age the number was reduced to 7.3×10^7 in the control population. However, when mice were treated with melatonin the cell number was $9.1x10^7$ at 24 months, showing that melatonin was able to arrest the fall in thymocyte numbers. In addition, it was demonstrated that melatonin prevents thymocyte apoptosis mediated by dexamethasone [107]. This protective effect of melatonin on thymocytes was attributed to its antiapoptotic action on thymus [108]. This reversal of age-associated thymic involution by melatonin add further support to the findings that melatonin can be used as a potential therapeutic agent for correcting the immunodeficiency state associated with aging and other immune-compromised states [8]. There is much debate as to whether melatonin favours Th-1 response (proinflammatory) or Th-2 response (anti-inflammatory). The Th-1/Th-2 balance is significant for an appropriate immune response [109]. Melatonin when administered to mice caused the release of pro inflammatory T helper (Th)-1 cytokines such IFNγ and IL-2, but when injected to antigen primed mice, it increased the production of IL-10, indicating that melatonin can activate anti inflammatory Th-2 immune response [110]. The relevance of Th-1 vs. Th-2 cytokine expression plays a crucial role in the regulation of cellular immune response [111]. In turn, these responses play a critical role in determining the susceptibility to infectious diseases and progress of inflammatory disorders [100, 112, 113]. Decreased serum levels of melatonin and IL-12 in a cohort of 77 HIV infected individuals has been reported, pointing to the possibility that impairment of immune response may have been a causal factor for reported decrease of serum melatonin levels [114]. Melatonin favours Th-1 lymphocyte response in immunodeficiency conditions [115]. It is suggested that Th-1 responses are readily transformed into Th-2 dominance through depletion of intracellular glutathione (GSH) [116]. GSH in reduced form is the most important cellular anti-oxidant. Depletion of GSH from antigen presenting cells *in vivo* caused reduced Th-1 activity and higher Th-2 activity [117]. The presence of high levels of oxidized glutathione resulted in polarization to type Th-2 cells [117]. Thus, the immune activity status can have either Th-1 or Th-2 characteristics, depending on the relative antioxidant status of the cells. Melatonin increases cytokine production like IL-2, IL-6, and IFN-γ in Th-1 cells and thereby enhances NK cell activity [118]. More recently melatonin has been shown to promote a time-dependent decrease of RORα levels suggesting a role for the RORα transcriptional activity. Interestingly RORα acts as a "molecular switch" implicated in the mutually exclusive generation of Th1 and T_{reg} cells, both involved in harm/protection balance of immune conditions such as autoimmunity or acute transplant rejection. Therefore the identification of melatonin as a natural modulator of RORα gives it a tremendous therapeutic potential for a variety of clinical disorders [119].

Melatonin, Immune Mechanism and Cytokines

Melatonin influences immune mechanism by regulating the production of cytokines from immunocompetent cells and it enhances the production of IL-2, IL-6, and IFNγ from cultured human mononuclear cells [118, 120, 121]. By activating monocytes, melatonin increases the production of IL-1, IL-6, TNF-α and reactive oxygen species [118]. Melatonin causes up-regulation of gene expression of tumor growth factor (TGF)-β, M-CSF, TNF-α, stem cell factor (SCF), IL-1β and IFNγ which in turn increases their production [113]. Melatonin's immunoenhancing effects are attributed to its antiapototic action on certain cells, antioxidant actions and on its influence in enhancing the production of cytokines [8]. A schematic diagram showing the beneficial effects on melatonin on immune mechanisms (both innate immunity and adaptive immunity) is presented in Fig. (**2**).

Melatonin's Effects on Infections and Immune Mechanism: Direct Evidences

Injections of melatonin to Siberian hamsters 4 h before the onset of darkness (so as to lengthen the endogenous melatonin profile) mimicked the effects of short days on febrile response to a simulated infection, emphasizing the importance of the duration of melatonin secretion in controlling host response to infection [122]. In another study on Siberian hamsters, it was noted that exposure to short days increases the number of circulating leukocytes and several

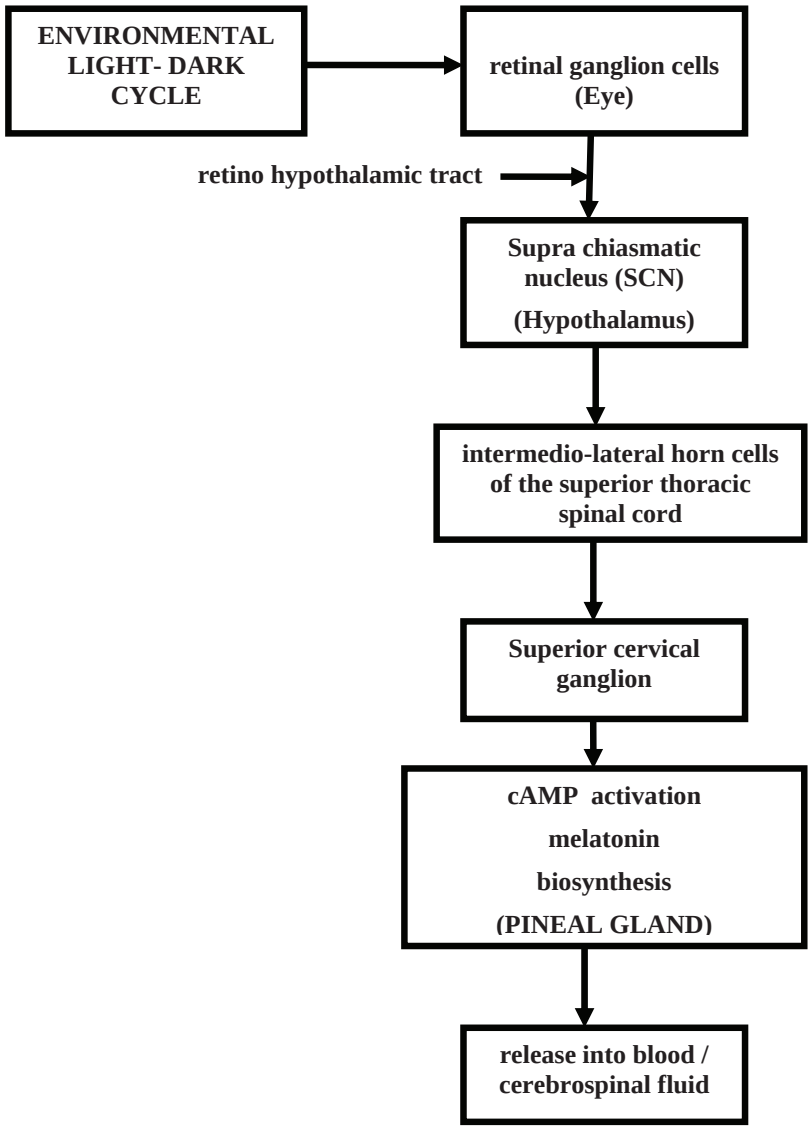

Fig. (2). Schematic diagram showing pathways regulating melatonin synthesis by the pineal gland.

lymphocyte populations. The short day exposure also attenuates both the magnitude and the duration of two major consequences of bacterial infection namely anorexia and cachexia [122]. As this effect of the short photoperiod on circulating leukocyte count was abolished by pinealectomy, it was concluded that pineal hormone melatonin was responsible for attenuating the adverse responses to bacterial infection [123].While melatonin has beneficial role in combating bacterial and viral infections through its immunomodulatory mechanisms has been already discussed, its metabolite in the neural tissue namely AFMK, is also effective in stimulating neutrophils and fighting against infections [124, 125]. Both melatonin and AFMK inhibit the release of IL-8 from neutrophils, but AFMK is much more effective than melatonin in this aspect. Moreover, the production of TNFα by neutrophils is also inhibited by melatonin. Since these substances (IL-8 and TNFα) contribute to inflammation [126], the inhibition of them by melatonin and AFMK is essential for combating against chronic inflammation [125].

Photoperiod, Melatonin Secretion, Immune Function and Seasonal Infections

Changing seasons exert fundamental effects on immune function and melatonin has a role in this aspect [127-130]. In mammals, the association between the environmental light cycle and immune response has been studied and it was shown that a short photoperiod (8 h of light and 16 h of darkness) has a stimulating effect on humoral immunity than a long photoperiod (16 h of light and 8 h of darkness). The photoperiodic timing of seasonal physiology in mammals depends upon entrainment of the suprachiasmatic nucleus (SCN) of the hypothalamus by the light-dark cycle which is coded by changes in the amount and duration of melatonin secretion, and is designated by the term "***encryption***" of the photoperiodic time cycle [131]. In this context, it is worth to have a brief understanding of the regulation of pineal melatonin production and secretion. Pineal melatonin biosynthesis and secretion has a circadian rhythm with low levels of

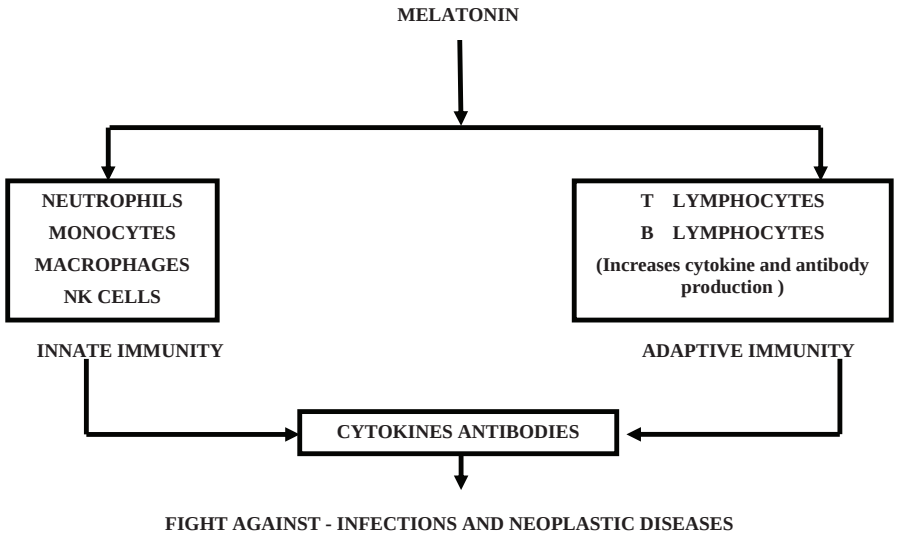

Fig. (3). Melatonin modulation of innate and adaptive immune mechanisms. nk, natural killer.

production during daytime and high levels at night-time. This circadian rhythm of melatonin production and secretion is regulated by a complex neural network, beginning with fibres originating in the retina, passing through the retino-hypothalmic tract to the SCN of the hypothalamus [132]. A subtype of retinal ganglion cells expressing the photopigment melanopsin are involved in transducing light information of the environment to the pineal gland through the SCN [133, 134]. Projections from the SCN pass through the paraventricular nucleus of the hypothalamus, medial forebrain bundle, and reticular formation to the intermediolateral horn cells of the spinal cord, that constitute the preganglionic sympathetic neurons innervating the superior cervical ganglion (SCG). From SCG post ganglionic sympathetic fibres arise and terminate on the pinealocytes, regulating the synthesis and secretion of melatonin. A diagram showing the path of light information from environment to SCN and pineal gland, involved in the regulation of melatonin synthesis is shown in Fig. (**3**). In addition to light-dark cycle of the day, seasonal changes in light dark cycle affect the amplitude and duration of melatonin secretion [132]. Seasonal variations in circadian rhythms of sleep and body temperature have been documented [135]. In a study conducted on young Japanese women exposed to naturally occurring day light, changes in plasma melatonin levels during various seasons has been documented. Peak melatonin levels and its duration are reported throughout the year as follows: **WINTER**: 15.80±8.18 pg/ml plasma: 4.05±81 minutes; **SPRING**: 15.22±3.38 pg/ml of plasma: 3.25±118 minutes; **SUMMER:** 21.46±10.52 pg/ml of plasma; 3.37 h ± 85 minutes; **AUTUMN:** 97.60±67.56 pg/ml of plasma; 5.31 h±75 minutes. Highest levels are reported during autumn season in this Japanese study. This study conducted by Ueno-Towatari *et al.* [136] shows that progressive shortening of the duration of day light is associated with increased nocturnal melatonin secretion during autumn season, supporting the influence of seasonal cycles on pineal melatonin secretion. In European hamsters, the nocturnal peak of pineal melatonin secretion during long naturally occurring photoperiods in summer is five times greater than daytime values, with duration of 4 h. By contrast, during short day periods of winter, nocturnal melatonin secretion is 15 times higher than daytime values with duration 9 h, showing thereby that the duration and amplitude of melatonin secretion correlates with changing photoperiods of the season [136]. The effect of short photoperiods on bacterial infections and pineal gland's role has been already discussed. Exposure of Siberian hamsters to short photoperiod attenuated adverse reactions to bacterial infections and this effect was abolished by pinealectomy, supporting the link between photoperiod-pineal gland and seasonal infections [123]. Seasonal-dependent immune mechanism were investigated in a double blind, placebo controlled trial conducted on human volunteers in the former Soviet Union. Intranasal administration of live influenza virus to 360 volunteers in Lenningrad during the month of January resulted in febrile reactions in 6.7% of the individuals, whereas administration of live influenza virus during the month of June to 197 volunteers resulted in febrile response of 0.8% [137].

Melatonin, Immune Mechanism and Anticarcinogenic Effects

Endogenous mechanisms protect against the development of carcinogenesis and they can be categorized into immune and non-immune dependent mechanisms. Immunosurveillance is one the major processes by which cancerous cells are detected and destroyed. NK cells play an important role in immune-surveillance against neoplasia and virus infected cells. The influence of melatonin on NK cell number and functional activity has already been discussed. The activation of monocytes and macrophages by melatonin is another mechanism through which melatonin exerts its anticarcinogenic effects. In addition to this melatonin's stimulatory effect on $CD4^+$ cells, Th-1 cells are also important for melatonin's anti-tumoral effects. Th-1 cells have the ability to kill the tumor cells by releasing cytokines that activate "death receptors" on tumor cell surface [138]. In addition to its effects of activating immune mechanisms, melatonin also has the ability to directly inhibit the cancer growth. This has been demonstrated in a recent study on hepatocarcinoma

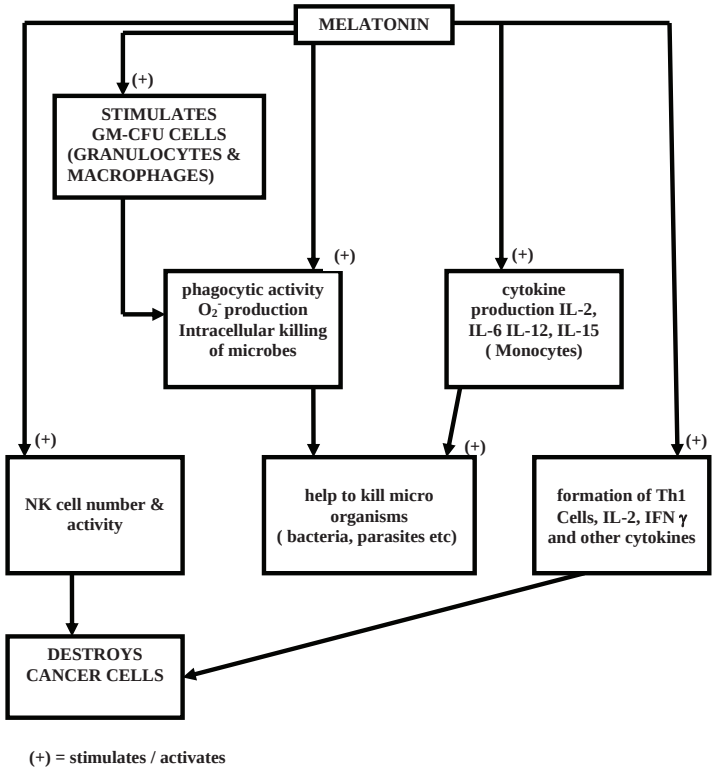

Fig. (4). Melatonin's activation of immune mechanisims in combating infectious and neoplastic diseases. GM-CFU, granulocyte macrophage colony-forming unit; O_2^-, superoxide; IL, interleukin; NK, natural killer.

HepG2 cell lines in which melatonin addition induced cell cycle arrest [139-141]. Melatonin's anti-carcinogenic effects through immune and non-immune mechanisms has been reviewed and published elsewhere and interested readers can refer to this paper [142].The combination of melatonin with immunotherapy on cancer patients has been tried in some studies. In a study conducted on 24 patients with advanced cell tumors (lung cancer-9 patients; colorectal cancer-7 patients; gastric cancer-3 patients; breast cancer-2 patients, hepatocarcinoma-1 patient, pancreatic cancer-1 patient, unknown tumor-1 patient) melatonin was given along with IL-2. Melatonin was administered 7 days before the injection of IL-2. Progress was found only in 6/24 patients, while stability was reported in 14/24 patients. This study shows melatonin combination with IL-2 in treating conditions with advanced neoplasms [142]. Similarly, administration of low subcutaneous dose of IL-2 with melatonin was found beneficial in causing tumor regression and prolonging the survival of cancer patients with metastatic colorectal cancer [143].The interrelationship between melatonin and immune mechanism in cancer was also studied in 42 patients with advanced GI cancer (colorectal, pancreatic, gastric cancer). The circadian rhythm of plasma melatonin is altered with peak melatonin level reaching at 0800-0900 h with 5-7 h delay with respect to average peak time seen in healthy controls. The study of TNF-α rhythm and soluble TNF-α receptors (type-1 and type-2) in these patients indicated an interrelationship between neuroendocrine network and cytokine network [144]. Based upon the neurohormonal studies carried out on lung cancer patients, in which overall increased serum levels of cortisol and altered cortsol rhythm was found, it was suggested that chronic treatment of advanced cancer patients with melatonin can normalize cortisol rhythm and this effect could stabilize the disease [57]. As melatonin possesses a strong antiapoptotic property, it can play an important role as a system regulator in immunoenhancement and has a potential value to be used as an adjuvant tumor immunotherapeutic agent [145].

CONCLUSIONS

Higher incidence of infectious diseases and neoplastic diseases are more common in elderly individuals. There is also seasonal outbreak of some infectious diseases. Our body has the ability to combat the infectious and neoplastic diseases and in this aspect, our immune mechanisms play a major role. Both innate and adaptive immunity have distinct roles. The greater incidence of infectious diseases and neoplastic diseases seen in the elderly is attributed to many factors like the nutritional status of the individual and environmental conditions; but most importantly, the immune status of the elderly plays a key role. As age advances there is drastic reduction in immune mechanisms of the body, known as immunosenescence which is said to be the main reason for greater occurrence of these diseases in the elderly. Immune mechanisms are regulated by a complex neuroendocrine network in which the hormone melatonin is very important. Melatonin has stimulatory role on innate immunity, cellular and humoral immunity. Experimental studies on various animals have shown the beneficial role of melatonin in immune modulation. The anti-carcinogenic effect of melatonin is exerted partly through its immunomodulatory mechanism. Recent studies on lung cancer patients suggest that there exist disordered hormonal secretion pattern and circadian variations of various lymphocyte subpopulations revealing

impaired integration among nervous, endocrine and immune systems in neoplastic disease Treatment with melatonin can set right this disturbed rhythms seen in cancer patients and can be of therapeutic value as "Chronotherapeutic agent" for treatment of cancer. Besides because of its antiapoptotic nature with immunoenhancement properties, it has a potential value for being used as an adjunct tumor immunotherapeutic agent. In addition, the photoperiodic changes of melatonin secretion and its correlation with seasonal infections are also gaining much attention. The synthesis of melatonin by lymphocytes and thymus supports the immune-modulatory role of melatonin. A schematic diagram showing the melatonin's activation of immune mechanisims in combating infectious and neoplastic diseases is summarized in Fig. (**4**). Analysis of these relationships points to the possibility of using melatonin as an adjunct therapy in the control and management of infectious diseases and cancer.

ABBREVIATIONS

AAAT	=	Arylakylamine-N-Acetyltransferase
AFMK	=	N^1-acetyl-N^2-formyl-5-methoxykynuramine
ASMT	=	Acetyl Serotonin Methyl Transferase
CCL	=	Chemokine Ligand
COX2	=	Cyclooxygenase-2
CSF	=	Cerebrospinal Fluid
DPSCs	=	Dental Pulp Stem Cells
GH	=	Growth Hormone
GSH	=	Glutathione
hESCs	=	Human Embryonic Stem Cells
HIV	=	Human Immunodeficiency Virus
hMSCs	=	Human Mesenchymal Stem Cells
IFN	=	Interferon
Ig	=	Immunoglobulin
IL	=	Interleukin
NK	=	Natural Killer
O_2	=	Anion Superoxide
OSCSCs	=	Oral Squamous Carcinoma Stem Cells
ROR	=	Retinoic Acid Receptor-Related Orphan Receptors
SCF	=	Stem Cell Factor
SCG	=	Superior Cervical Ganglion
SCN	=	Suprachiasmatic Nucleus
TCR	=	T Cell Receptor
TGF	=	Tumor Growth Factor
Th	=	T Helper
TRH	=	Thyrotropin Releasing Hormone
TSH	=	Thyroid Stimulating Hormone

CONFLICT OF INTEREST

V. Srinivasan, Founder & Chairman, Sri Sathya Sai Medical Educational and Research Foundation, Prasanthi Nilayam, 40- Kovai thirunagar, Coimbatore-641014, Tamilnadu, INDIA,declares no competing interest that might be perceived to influence the contents of the paper. No funding or fees has been received from any source for preparation, or submission of the paper. Sri Sathya Sai Medical Educational and Research Foundation (SSSMERF) is not funded from any organization with any political, communal, religious, commercial, academic, ideological or intellectual interests. The Corresponding author (VS) has not received any salary, fees or funding during the past three years ever since the SSSMERF was established in 2009. All other authors of this paper also declare that they have no propriety, professional or any other type of personal interest of any kind in any product or services and/or company that could be construed or considered as a potential conflict of interest that might have influenced the views expressed in this paper. All other authors also declare that they have no conflict of interest.

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