Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

Pinealectomy alters IFN-γ and IL-10 levels in primary thymocyte culture of rats

Zafer Sahin[1*], Suleyman Sandal[2], Bayram Yilmaz[3], Ozgur Bulmus[4] Gokcen Ozdemir[4], Selim Kutlu[5], Ahmet Godekmerdan[6], Haluk Kelestimur[4]

[1] Department of Physiology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey
[2] Department of Physiology, Faculty of Medicine, Inonu University, Malatya, Turkey
[3] Department of Physiology, Faculty of Medicine, Yeditepe University, İstanbul, Turkey
[4] Department of Physiology, Faculty of Medicine, Firat University, Elazig, Turkey
[5] Department of Physiology, Faculty of Meram Medicine, Necmettin Erbakan University, Konya, Turkey
[6] Deparment of Microbiology, Faculty of Medicine, Ankara Yildirim Beyazit University, Ankara, Turkey

Correspondence to: zafersahin@ktu.edu.tr, zafersahin55@yahoo.com

Received November 26, 2017; **Accepted** November 12, 2018; **Published** November 30, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.14.5

Abstract: Melatonin, produced mainly by the pineal gland, has an immunomodulatory role. However, the effects of the pineal gland and/or melatonin on thymus cytokine levels such as interferon-gamma (IFN-γ), interleukin (IL)-4, and IL-10 are not well known. Twenty-one male Wistar rats (220-250 gr) were randomly divided into three groups (n=7): intact control, sham, and pinealectomy. Primary thymocyte cultures were prepared from each group and dispensed into well plates as Control, DMSO (or vehicle), Sham-pinealectomy, Pinealectomy, Pinealectomy+10μM melatonin, and Pinealectomy+100μM melatonin. IFN-γ, IL-4, and IL-10 concentrations were measured in the thymocytes (as nonstimulated and Concanavalin A-stimulated) after 24 h. IFN-γ levels significantly increased and IL-10 levels significantly decreased in both media prepared from pinealectomized rats. There was no significant difference between the groups in terms of IL-4. In the pinealectomy+10μM melatonin group, IFN-γ and IL-10 levels did not differ from the pinealectomy group. However, the dose of 100μM melatonin caused a decrease in levels of IFN-γ in both thymocyte media and an increase in the concentration of IL-10 in Concanavalin A-stimulated thymocytes. In conclusion, pineal gland and/or melatonin affect IFN-γ and IL-10 levels in the thymus gland.

Key words: Pineal gland; Melatonin; Cytokine; Thymocyte; T helper; Rat.

Introduction

Melatonin is a neuroendocrine hormone that is secreted by the pineal gland as well as the retina, gastrointestinal tract, and immune-competent structures containing bone marrow (1), lymphocytes (2), and thymus gland (3). Besides playing an essential role as a photoperiodic signal, melatonin plays a regulatory role in a range of other essential physiological mechanisms, including the adjustment of reproductive, antioxidant, neuroendocrine and, immunological functions (4, 5).

In the last four decades, various experiments have revealed the interaction between the immune system and pineal gland and/or melatonin (6). Pinealectomy or any different procedures such as experience to continuous lighting, which restrains the melatonin production, weakens both the cellular and humoral immune system. These alterations are partly modified by the melatonin administrations (7, 8). It is known that pinealectomy, in connection with the thymus gland, leads to a reduction in the weight and structural organization of thymus (9-11) and such alterations can be restored by exogenous melatonin treatment (11). Moreover, expression of melatonin receptors in the thymus gland joins the evidence of the connection between the immune system and pineal gland (12-14).

The thymus gland is a primary lymphoid structure in which bone marrow-originated T cell precursors complete a gradual course of maturation (15, 16). Fully matured T cells leave the thymus and seed the peripheral immune structures such as lymph nodes, tonsils, spleen, and Peyer's patches (15). This process starts at birth and continues throughout life. In a proper cytokine environment, activated CD4+ T cells differentiate into seven distinct subsets: Th1, Th2, Th9, Th17, Th22, Treg, and T follicular helper (Tfh) cells (17). Th1 cells predominantly produce IL-2, IFN-γ, and tumor necrosis factor-alpha (TNF-α), while Th2 cells predominantly secrete IL-4, IL-5, IL-10, and IL-13 (17, 18). Moreover, Th1 lymphocytes manage the cellular immune activity to combat intracellular antibody producing structures. Th2 lymphocytes conduct the humoral response to capture extracellular pathogens (19).

There is evidence that the pineal gland and melatonin administration exert an immunomodulatory effect in both *in vivo* and *in vitro* models such as splenocytes and peripheral blood mononuclear cells (PBMC). Although *in vivo* models show that melatonin can be considered as a positive regulator of immune responses, the results are less clear when melatonin is used *in vitro* (20). Moreover, the effects of the pineal gland or melatonin on thymus cytokine levels are not well known. Therefore, we aimed to evaluate the effects of pinealectomy and *in vitro* melatonin treatment on IFN-γ, IL-4, and IL-

10 levels in the primary thymus cell cultures prepared from male rats.

Materials and Methods

Animals and drugs

Adult Wistar albino male rats (each weighing around 220-250 g) were provided from the Experimental Research Center of Firat University (Elazig, Turkey). The animals were housed under standard light/darkness schedule (12 h in light/12 h in dark from 07:00 each day), at constant temperature (21±1°C) and humidity (55±5%) with free access to pelleted food and fresh water. The experimental protocols were approved by the Ethical Committee of Firat University, and the rats were treated in accordance with the national and international laws and policies on the care and use of laboratory animals.

Experimental design

Rats were randomly distributed into three groups (n=7 for each group). In Group I (intact control), the rats in this group did not undergo surgery or drug treatment. In Group II (sham-pinealectomy), rats were surgically operated only, but their pineal glands were not removed. This group was further divided into two groups as dimethyl sulfoxide (DMSO) and sham-pinealectomy in the well plates. In Group III (pinealectomy), rats underwent surgical pinealectomy. This last group was also further divided into three subgroups as pinealectomy, pinealectomy+10 μM melatonin, and pinealectomy+100 μM melatonin in a 96-well plate. The IFN-γ, IL-4, and IL-10 levels were measured in a total of six groups in the well plates.

Pinealectomy operation

The pinealectomy operation was performed according to a method we performed previously (21). Surgery was performed under general anesthesia with a xylazine (10 mg/kg)/ketamine (90 mg/kg) cocktail. Then, each rat was placed on a stereotaxic apparatus (Stoelting Co, Illinois, USA). After a haircut, the scalp was cleaned with iodine solution, and an approximately 1.75 cm incision was made in which the middle line was cut longitudinally until the occipital bone. Periosteum, the area between the sagittal and lambdoid sutures, was scraped from the temporal muscles and membrane with the help of a scalpel. Oval skull fragment, which was approximately 3-4 mm in diameter, was drilled by a dental drill on the right side of the intersection of the transverse and sagittal sinus commissure. Brain membrane was carefully pierced with a fine-tipped, 60-degree angle tweezers, and was advanced approximately 3-5 mm horizontally inside the area. After capturing the pineal stalk with a fine tweezers, the gland was ruptured and taken out. In next step, the skin was sutured with 4/0 silk suture.

The sham-pinealectomy operation was performed by the same method, but the pineal gland was not removed. For amelioration, rats were maintained in an appropriate environment for two weeks.

Preparation of primary thymocyte culture

Primary thymocyte cultures were prepared according to the method of Oyama *et al*. (22) with some modifications (23,24). Thymus glands of rats were surgically dissected and thymocytes were gently diluted in RPMI-1640 solution (Biological Industries, Israel) utilizing two parts of cold glass bars. In order to obtain a single cell suspension, the tissue residues were purged off with clamps and medical scissors. Thymocytes were washed twice with RPMI-1640. Then, the cells were diluted with RPMI-1640 media combined with 2 mM L-glutamine (Sigma, St. Louis, MO, USA), 20 mM HEPES (Biological Industries, Israel), 10% fetal calf serum, and 1% penicillin-streptomycin (Mediatech Collegro VA, USA) to 1×10^6 cell/ml in the 10 μg/ml Concanavalin (Con) A (Sigma, St. Louis, MO, USA) induced thymocytes medium. After that, a 1 ml cell population was distributed into the well plates (TRP, Switzerland), and thymocytes of the pinealectomy group were administrated 10 and 100 μM doses of melatonin (Sigma, St. Louis, MO, USA). Melatonin dissolved in DMSO. The cells were incubated in a humidifier incubator (Hera Cell, Germany) at 37°C and 95% O_2 - 5% CO_2 for 24 h. Thymocyte quantities determined by checking for cell viability at the end of each application term in all groups. Our monitoring demonstrated that the thymocytes counts at the end of the application were very similar to the initial population of 1×10^6 cells/ml. These verify the result that the variations in cytokine secretion ratios ascertained an effect of the exogenous melatonin, which cannot be attributed to a probable change in the thymocyte counts. Supernatants were collected 24 h after the administration of 10 and 100 μM doses of melatonin.

Cytokine assay

IFN-γ, IL-4, and IL-10 levels were detected in the thymocyte supernatants using commercial rat ELISA kits (BioSource International Inc, Camarillo, California, USA) that were utilized in conformity with the manufacturer's explanations, and absorbance ratios were detected at 450 nm, using an ELISA plate-reader (BIOTEC Instruments, Inc. Vermont, USA).

Statistical analysis

All results were expressed as mean ± SEM. The Shapiro-Wilk test was used in all cases to test for normality of the data set, the homogeneity of variances was evaluated using Levene test and the results were found to be nonparametric. Therefore, data were analyzed by Kruskal-Wallis test followed by Mann-Whitney U-test. SPSS 10.1 for Windows was utilized for statistical analyses and $p< 0.05$ value was accepted to be statistically significant.

Results

Effects of pinealectomy and different doses of melatonin on IFN-γ levels

Figure 1 (A and B) presents the effects of pinealectomy and treatment with different doses of melatonin after pinealectomy on IFN-γ levels in the nonstimulated (Figure 1A) and Con A-stimulated thymocytes (Figure 1B), detected 24 h after the application. IFN-γ levels did not differ among the intact control, DMSO and sham-pinealectomy groups. Therefore, comparisons were made

according to the values of the intact control group. The pinealectomy caused significant increases in the IFN-γ levels (5.44±0.41 and 15.06±0.27 pg/ml in the nonstimulated and Con A-stimulated thymocytes, respectively) compared to the control group (3.65±0.33 and 11.04±0.79 pg/ml, respectively, $p<0.05$). When the cells were treated with 10 and 100 µM concentration of melatonin after pinealectomy, IFN-γ levels of the pinealectomy+10 µM melatonin group (5.43±0.32 and 14.45±0.28 pg/ml, respectively) were significantly higher than the control group in both thymocyte medium ($p<0.05$). Administration of 100 µM melatonin did not significantly alter the IFN-γ level in the pinealectomy+100 µM melatonin (3.93±0.05 and 12.35±0.92 pg/ml, respectively) group compared with the control. However, the IFN-γ level of the pinealectomy+100 µM melatonin group was significantly lower than the IFN-γ level in the pinealectomy group in both thymocyte medium ($p<0.05$).

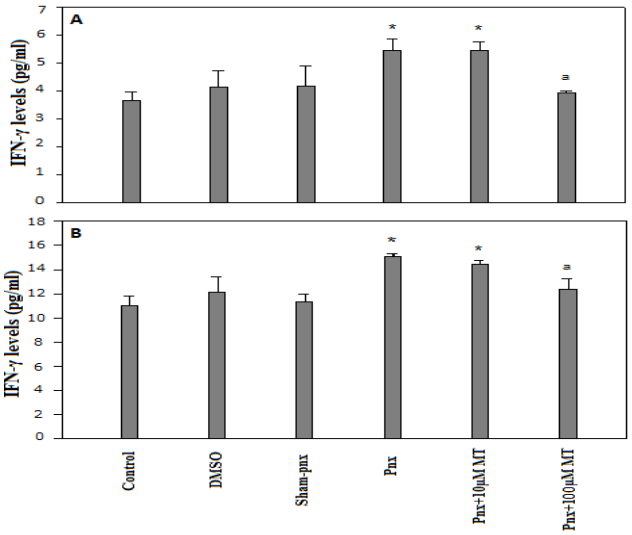

Figure 1. Changes in IFN-γ levels (mean±SEM) of rat primer thymocyte cultures in the non-stimulated (A) and Con A-stimulated (B). *: $p<0.05$ compared to control, a: $p<0.05$ compared to pinealectomy group. Kruskal-Wallis test followed by Mann-Whitney U-test was used. IFN-γ: interferon-gamma, DMSO: Dimethyl sulfoxide, Pnx: pinealectomy, MT: melatonin.

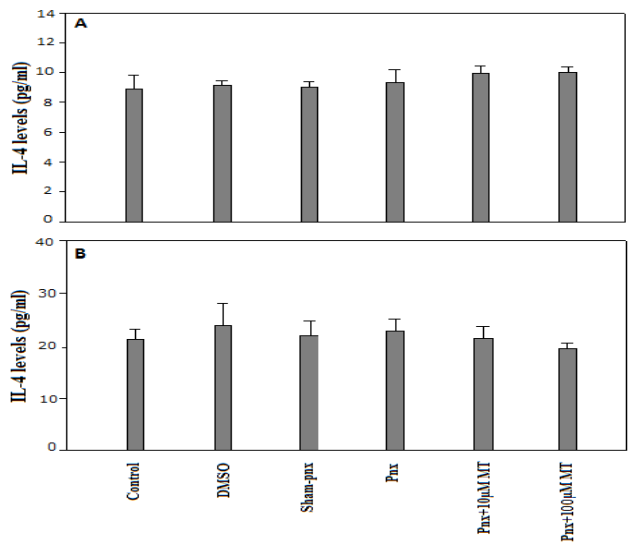

Figure 2. Changes in IL-4 levels (mean±SEM) of rat primer thymocyte cultures in the non-stimulated (A) and Con A-stimulated (B). IL-4: interleukin-4, DMSO: Dimethyl sulfoxide, Pnx: pinealectomy, MT: melatonin.

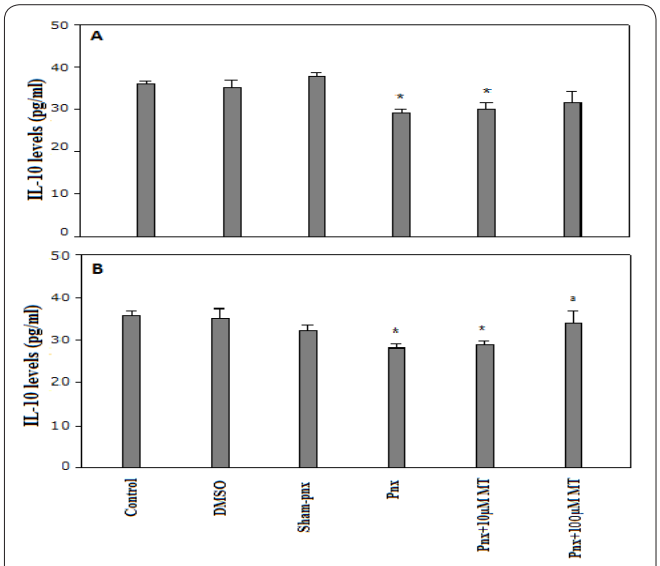

Figure 3. Changes in IL-10 levels (mean±SEM) of rat primer thymocyte cultures in the non-stimulated (A) and Con A-stimulated (B). *: $p<0.05$ compared to control, a: $p<0.05$ compared to pinealectomy group. Kruskal-Wallis test followed by Mann-Whitney U-test was used. IL-10: interleukin, DMSO: Dimethyl sulfoxide, Pnx: pinealectomy, MT: melatonin.

Effects of pinealectomy and different concentrations of melatonin on IL-4 levels

The effects of pinealectomy and treatment with different doses of melatonin after pinealectomy on IL-4 production in the nonstimulated and Con A-stimulated thymocytes are summarized in Figure 2 (A and B). Pinealectomy and melatonin administrations did not produce any remarkable change in the IL-4 level of the groups compared to those of the control group in both thymocyte medium.

Effects of pinealectomy and different concentrations of melatonin on IL-10 levels

Figure 3 (A and B) summarizes the effects of pinealectomy and melatonin after pinealectomy on the IL-10 level in the nonstimulated (Figure 3A) and Con A-stimulated thymocytes (Figure 3B). IL-10 levels did not differ among the intact control, DMSO and sham-pinealectomy groups. Therefore, comparisons were performed according to the values of the intact control group. Pinealectomy caused a significant decline in the IL-10 concentrations in the non-stimulated and Con A-stimulated thymocytes (29.18±1 and 27.93±0.98 pg/ml, respectively) compared to those in the control group (36.10±0.72 and 35.48±1.45 pg/ml, respectively, $p<0.05$). The IL-10 levels of pinealectomy+10 µM melatonin group (30.24±1.47 and 28.77±0.85 pg/ml) were significantly lower than the control group in both medium ($p<0.05$). The IL-10 concentration did not differ between the pinealectomy+100 µM melatonin group (31.81±2.49 and 33.66±3.21 pg/ml, respectively) and control group, but its level was found to be significantly higher in the pinealectomy+100 µM melatonin group compared to the pinealectomy group in the Con A-stimulated thymocytes ($p<0.05$).

Discussion

The results of the present study indicate that pinealectomy significantly increased the IFN-γ production

and significantly decreased the IL-10 release but it did not change the IL-4 levels in the nonstimulated and Con A-stimulated thymocytes.

In recent years, accumulated evidence indicates that neuroendocrine signals from the brain interweave with the immune system (25). As a sign of this interaction, the thymus gland of both human and animal expresses melatonin receptors (12, 13, 26). The peak nocturnal melatonin secretion from the pineal gland declines with age (27). Administering exogenous melatonin can prevent old thymus cells from apoptosis both *in vivo* and *in vitro* conditions (28, 29). Our results in the pinealectomy group with an increase in the IFN-γ levels and a decrease in the IL-10 levels may be associated with these interactions. Reduction of melatonin after pinealectomy is likely to have formed based on the hormone secretion profile of the pineal gland due to aging. Because the aging process consociates with a low-chronic stage, inflammation is induced by an exchange from the fine stability of Th1 and Th2 cytokines to a pro-inflammatory (Th1) phase (30).

In vitro melatonin administration had a dose-dependent effect on the thymocyte medium prepared from pinealectomized rats. Administration of 10μM melatonin in the nonstimulated and Con A-stimulated thymocytes caused significant increases in the IFN-γ levels and decreases in the IL-10 levels. However, the IL-4 concentrations were unchanged in both thymocyte media. When 100μM melatonin was administered to the nonstimulated and Con A-stimulated thymocytes, the IFN-γ, IL-10 and IL-4 levels were not significantly different from the control group. However, when the pinealectomy+10μM melatonin and pinealectomy+100μM melatonin groups were compared to the pinealectomy group, it was observed that 100 μM dose of melatonin significantly reduced the IFN-γ levels in both thymocyte media. The IL-10 level in the nonstimulated thymocytes was significantly higher than the pinealectomy group only. Thus, our results indicate that the high dose of *in vitro* melatonin exert a modulating effect on the pinealectomy-induced alterations in the IFN-γ and IL-10. This finding does not seem to be consistent with the general idea that melatonin enhances the IFN-γ production (31, 32). However, Park and Chun (33) reported that 10 μM melatonin can effectively modulate LPS-induced proinflammatory mediators and phagocytic activity of activated BV2 microglia. The fact that high melatonin dose caused a reduction in the IFN-γ production may have resulted from a decrease in the melatonin receptor expression related to the phenomena of downregulation. In our experiments, the possibility of such interactions remains a question, because we treated *in vitro* melatonin only to the pinealectomized groups. Jimenez-Jorge et al. (26) suggested that melatonin content of the thymus gland was higher in pinealectomized rats than sham-pinealectomized rats. In general, while extrapineal melatonin may play an autocrine and paracrine role in the body structures where it is synthesized, pineal melatonin appears to play a regulatory role as an endocrine signal in the body by exerting a neuroendocrine activity connecting its target cells via the blood circulation (20, 34, 35). These data indicate that the pineal gland is an essential regulator of melatonin synthesis in the thymus gland and other body areas. However, according to our results, it is not possible to explain these interactions because the melatonin levels are not evaluated in plasma or in thymocyte media.

Our experimental design may be another important factor in this context because most others studies were conducted on different cell types such as splenocytes, peripheral blood mononuclear cells (PBMC), and plasma or serum. The effect of melatonin on PBMC was investigated by Garcia-Mauriño et al. (31), who observed that melatonin was able to activate human Th1 lymphocytes by increasing the generation of IL-2 and IFN-γ. Therefore, the data we collected on the effect of pinealectomy and *in vitro* melatonin on IFN-γ levels seem to contradict the results of earlier studies. Regarding the Th2 cytokines, it has been reported that melatonin has a negative correlation with the IL-10 production in humans (36), whereas many researchers claimed that this hormone does not affect IL-4 production (31, 36). Interestingly, melatonin can enhance Th2 activities in such a way that it not only stimulates the secretion of IFN-γ and IL-2 but also of IL-10 (37).

It is not well known, whether the characteristics of the cytokine expression in the peripheral immune structures such as lymph nodes, tonsils, spleen, and payer's patches PBMC or other cells thymocyte are different from each other or not. It has been reported that some differences may be in the function of the cytokine system located in the thymus and peripheral compartments of the immune system (38). The functions of some cytokines in the thymus can be significantly different from those in the periphery of the immune system. For example, proinflammatory cytokines act in the thymus as factors or cofactors of thymocyte. In addition, they act in the activation, proliferation or differentiation of thymic epithelial cells. The key cytokines of Th1 and Th2 cells, IFNγ and IL-4, do not participate in the immune response but mediate the autoregulation of the thymocyte population. However, the thymus produces hormones and cytokines that regulate the immune function (39). The main producers of cytokines in the thymus are thymocytes and thymic epithelial cells, but all types of thymic cells are able to produce cytokines either spontaneously or after stimulation (38). A previous study noted that cytokine secretion by T lymphocytes has a vital role in mounting adaptive immune responses (40). The thymic production of Treg cells requires IL-2, and IL-4 that is synergistic with IL-2 in the induction of thymocyte proliferation in fetal thymic organ culture (41, 42). IL-10 produced by Treg cells, and other chronically stimulated T helper cells and B cells are important for maintaining immune homeostasis at mucosal surfaces and also contributes to immune suppression (43-45). IFN-γ activates thymic epithelial cells and increases surface expression of MHC classes I and II, and other membrane proteins (46). Furthermore, IFN-γ supports thymocyte differentiation through its effect on functions of thymic epithelial cells. These reports indicate that the functions of numerous cytokines in the thymus are not well understood. Understanding the effects of intrathymic cytokines may help to reveal some unknown aspects of thymic physiology (39). Unfortunately, there are not enough studies to picture the interaction of the pineal gland or melatonin with thymus cytokine production. Therefore, our results are important for contributing to

the thymic physiology.

In conclusion, pinealectomy caused an increase in the IFN-γ level and a decrease in the IL-10 level in the nonstimulated and Con A-stimulated thymocytes. Moreover, *in vitro* melatonin treatment revealed a modulating effect on these cytokines in a dose-dependent manner in pinealectomized rats. Our results indicate that the immunomodulatory role of pineal gland/melatonin includes the cytokine production of the thymus gland.

Acknowledgment
This work was supported by the Research Foundation of Fırat University (Project No: FÜBAP-1101).

Interest conflict
The authors declare no conflict of interest.

Author's contribution
Each author has made an important scientific contribution to the study and has assisted with the drafting of the manuscript.

References

1. Conti A, Conconi S, Hertens E, Skwarlo-Sonta K, Markowska M, Maestroni JM. Evidence for melatonin synthesis in mouse and human bone marrow cells. J Pineal Res 2000; 28:193–202.
2. Carrillo-Vico A, Lardone PJ, Fernandez-Santos JM, Martin-Lacave I, Calvo JR, Karasek M, et al. Human lymphocyte-synthesized melatonin is involved in the regulation of the interleukin-2/interleukin-2 receptor system. J Clin Endocrinol Metab 2005; 90:992–1000.
3. Naranjo MC, Guerrero JM, Rubio A, Lardone PJ, Carrillo-Vico A, Carrascosa-Salmoral MP, et al. Melatonin biosynthesis in the thymus of humans and rats. Cell Mol Life Sci 2007; 64:781–790.
4. Reiter RJ. Melatonin: the chemical expression of darkness. Mol Cell Endocrinol 1991; 79:153–8.
5. Brzezinski A. Melatonin in humans. N Engl J Med 1997; 336:186-95.
6. Csaba G. The pineal regulation of the immune system: 40 years since the discovery. Acta Microbiol Immunol Hung 2013; 60:77-91.
7. Guerrero JM, Reiter RJ. Melatonin-immune system relationships. Curr Top Med Chem 2002; 2:167–179.
8. Skwarlo-Sonta K. Melatonin in immunity: comparative aspects. Neuroendocrinol Lett 2002; 23:61–66.
9. Csaba G, Barath P. Morphological changes of thymus and the thyroid gland after postnatal extirpation of pineal body. Endocrinol Exp 1975; 9:59–67.
10. Vaughan MK, Reiter RJ. Transient hypertrophy of the ventral prostate and coagulating glands and accelerated thymic involution following pinealectomy in the mouse. Tex Rep Biol Med 1971; 29: 579–586.
11. Oner H, Kus I, Oner J, Ogeturk M, Ozan E, Ayar A. Possible effects of melatonin on thymus gland after pinealectomy in rats. Neuro Endocrinol Lett 2004; 25:115–118.
12. Pozo D, Delgado M, Fernandez-Santos JM, Calvo JR, Gomariz RP, Martin-Lacave I, et al. Expression of the Mel1a-melatonin receptor mRNA in T and B subsets of lymphocytes from rat thymus and spleen. FASEB J 1997; 11:466–473.
13. Rafii-El-Idrissi M, Calvo JR, Harmouch A, Garcia-Maurino S, Guerrero JM. Specific binding of melatonin by purified cell nuclei from spleen and thymus of the rat. J Neuroimmunol 1998; 86:190–197.
14. Carrillo-Vico A, Garcia-Perganeda A, Naji L, Calvo J R, Romero MP, Guerrero JM. Expression of membrane and nuclear melatonin receptor mRNA and protein in the mouse immune system. Cell Mol Life Sci 2003; 60:2272–2278.
15. Anderson G, Moore NC, Owen JJ, Jenkinson EJ. Cellular interactions in thymocyte development. Annu Rev Immunol 1996; 14:73–99.
16. Rothenberg EV, Moore JE, Yui MA. Launching the T-cell-lineage developmental programme. Nat Rev Immunol 2008; 8:9-21.
17. Raphael I, Nalawade S, Eagar TN, Forsthuber TG. T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine 2015; 74:5-17.
18. Vahedi GC, Poholek A, Hand TW, Laurence A, Kanno Y, O'Shea JJ, et al. Helper T-cell identity and evolution of differential transcriptomes and epigenomes. Immunol Rev 2013; 252:24-40.
19. Ren W, Liu G, Chen S, Yin J, Wang J, Tan B, et al. Melatonin signaling in T cells: Functions and applications. J Pineal Res 2017; 62,e12394.
20. Carrillo-Vico A, Guerrero JM, Lardone PJ, Reiter RJ. A review of the multiple actions of melatonin on the immune system. Endocrine 2005; 27:189–200.
21. Canpolat S, Sandal S, Yilmaz B, Yasar A, Kutlu S, Baydas G, et al. Effects of pinealectomy and exogenous melatonin on serum leptin levels in male rat. Eur J Pharmacol 2001; 428:145-8.
22. Oyama Y, Carpenter DO, Ueno S, Hayashi H, Tomiyoshi F. Methylmercury induces Ca2+-dependent hyperpolarization of mouse thymocytes. A flow-cytometric study using fluorescent dyes. Eur J Pharmacol 1995; 293:101–107.
23. Sandal S, Yilmaz B, Godekmerdan A, Kelestimur H, Carpenter DO. Effects of PCBs 52 and 77 on Th1/Th2 balance in mouse thymocyte cell cultures. Immunopharmacol Immunotoxicol 2005; 27:601-613.
24. Sandal S, Tuneva J, Yilmaz B, Carpenter DO. Effects of cholesterol and docosahexaenoic acid on cell viability and (Ca(2+))(i) levels in acutely isolated mouse thymocytes. Cell Biochem Funct 2009; 27:155-61.
25. Elenkov IJ. Neurohormonal-cytokine interactions: Implications for inflammation, common human diseases and well-being Neurochem Int 2008; 52:40-51.
26. Jimenez-Jorge S, Jimenez-Caliani AJ, Guerrero JM, Naranjo MC, Lardone PJ, Carrillo-Vico A, et al. Melatonin synthesis and melatonin-membrane receptor (MT1) expression during rat thymus development: role of the pineal gland. J Pineal Res 2005; 39:77–83.
27. Maestroni GJ. The immunoneuroendocrine role of melatonin. J Pineal Res 1993; 14:1–10.
28. Sainz RM, Mayo JC, Uria H, Kotler M, Antolin I, Rodriguez C, et al. The pineal neurohormone melatonin prevents in vivo and in vitro apoptosis in thymocytes. J Pineal Res 1995; 19:178–188.
29. Fabris N, Mocchegiani E, Provinciali M. Plasticity of neuro-endocrine-thymus interactions during aging-a minireview. Cell Mol Biol (Noisy-le-grand). 1997;43:529-41.
30. Vasto S, Candore G, Balistreri CR, Caruso M, Colonna-Romano G, Grimaldi MP, et al. Inflammatory networks in ageing, age-related diseases and longevity. Mech Ageing Dev 2007; 128:83–91.
31. Garcia-Maurino S, Gonzalez-Haba MG, Calvo JR, Rafii-El-Idrissi M, Sanchez-Margalet V, Goberna R, et al. Melatonin enhances IL-2, IL-6, and IFN-gamma production by human circulating CD4+ cells: a possible nuclear receptor-mediated mechanism involving T helper type 1 lymphocytes and monocytes. J Immunol 1997; 159:574-581.
32. Miller SC, Pandi-Perumal SR, Esquifino AI, Cardinali DP, Maestroni GJ. The role of melatonin in immuno-enhancement: Potential application in cancer. Int J Exp Pathol 2006; 87:81–87.
33. Park E, Chun HS. Melatonin Attenuates Manganese and Lipopolysaccharide-Induced Inflammatory Activation of BV2 Microglia. Neurochem Res 2017; 42:656-666.

34. Petrovsky N, Harrsion LC. Diurnal rhythmicity of human cytokine production: a dynamic disequilibrium in T helper cell type 1/T helper cell type 2 balance? J Immunol 1997; 158:5163-5168.
35. Motilva V, Cabeza J, Alarcón de la Lastra C. New issues about melatonin and its effects on the digestive system. Curr Pharm Des 2001; 7: 909–931.
36. Petrovsky N, Harrsion LC. Diurnal rhythmicity of human cytokine production: a dynamic disequilibrium in T helper cell type 1/T helper cell type 2 balance? J Immunol 1997; 158:5163-5168.
37. Raghavendra V, Singh V, Kulkarni SK, Agrewala JN. Melatonin enhances Th2 cell mediated immune responses: Lack of sensitivity to reversal by naltrexone or benzodiazepine receptor antagonists. Mol Cell Biochem 2001; 221:57-62.
38. Yarilin AA, Belyakov IM. Cytokines in the thymus: production and biological effects. Curr Med Chem 2004; 11:447-64.
39. Yan F, Mo X, Liu J, Ye S, Zeng X, Chen D. Thymic function in the regulation of T cells, and molecular mechanisms underlying the modulation of cytokines and stress signaling (Review). Mol Med Rep 2017; 16:7175-7184.
40. Chemin K, Bohineust A, Dogniaux S, Tourret M., Guégan S., Miro F., et al. Cytokine secretion by CD4+ T cells at the immunological synapse requires Cdc42-dependent local actin remodeling but not microtubule organizing center polarity. J Immunol 2012; 189:2159-2168.
41. Zlotnik A, Ransom J, Frank G, Fischer M, Howard M. Interleukin 4 is a growth factor for activated thymocytes: Possible role in T-cell ontogeny. Proc Natl Acad Sci USA 1987; 84:3856-3860.
42. Meilin A, Sharabi Y, Shoham J. Analysis of thymic stromal cell subpopulations grown in vitro on extracellular matrix in defined medium-V. Proliferation regulating activities in supernatants of human thymic epithelial cell cultures. Int J Immunopharmacol 1997; 19:39-47.
43. Barnes MJ, Powrie F. Regulatory T cells reinforce intestinal homeostasis. Immunity 2009; 31:401-411.
44. Shevach EM. Mechanisms of Foxp3+ T regulatory cell-mediated suppression. Immunity 2009; 30:636-645.
45. Mittal SK, Roche PA. Suppression of antigen presentation by IL-10. Curr Opin Immunol 2015; 34:22-27.
46. Patel DD, Whichard LP, Radcliff G, Denning SM, Haynes BF. Characterization of human thymic epithelial cell surface antigens: phenotypic similarity of thymic epithelial cells to epidermal keratinocytes. J Clin Immunol 1995; 15:80-92.