Thyroid Hormone Modulation of Immunity: Its Participation in Chronic Stress-Induced Immune Alterations

Ana M. Genaro[1,2], Alicia J. Klecha[1,2], Luciana R. Frick[1], Maria L. Barreiro Arcos[1] and Graciela A. Cremaschi[*,1,2]

[1]*Centro de Estudios Farmacológicos y Botánicos (CEFYBO) – Consejo Nacional de Ciencia y Tecnología (CONICET),* [2]*Laboratorio de Radioisótopos, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires (UBA), Buenos Aires, Argentina*

Abstract: The interaction between the immune and the neuroendocrine systems involves, in a bidirectional circuit, shared cellular receptors and their coupled signaling pathways. Disruptions of this circuit lead to pathological situations. Between the endocrine factors, thyroid hormones show to play a role in immunomodulation, maintaining immune system homeostasis in response to stress-mediated immunosuppression. Several experimental evidences showed that hypothyroidism leads to a depression of humoral and cell-mediated immune responses, effects that were reversed by restoration of the euthyroid state. By other hand, chronic stressful situations impair T-cell mediated immunity, affecting the intracellular signals involved in lymphocyte activation. Consequently, a reduction in both T-cell dependent antibody production and lymphocyte function were also described. Besides, a decrease in thyroid hormone serum levels was observed in these conditions. These stress-induced endocrine-immune alterations impact in tumor development, as an enhancement of tumor growth was described in animal models of chronic stress. Interestingly, hormone replacement treatment of chronic stressed animals, which restored the euthyroid status, reversed the observed reduction of T-cell responses improving the outcome of tumor bearing animals. These evidences strengthen the important role that thyroid hormones play in immunomodulation, with a special emphasis in their participation as neuroendocrine regulators of stress-mediated immune deficit.

Keywords: Thyroid hormones, stress, neuroendocrine system, immune system, tumor.

INTRODUCTION

The immune system reacts to the antigenic challenge with defensive responses designed to eliminate 'foreign' or 'non-self' material and returns to a standby or surveillance mode. For this purpose a fine and complex net of autorregulatory mechanisms exists that participates in homeostatic immune functioning to avoid harmful responses to self. Despite this functional autonomy, experimental evidences from the last 20 years have shown that the neuroendocrine and the immune systems are intimately linked and do not function as independent systems [1-3]. In fact, they are interrelated *via* a bidirectional network in which hormones and neurotransmitters affect immune function and, in turn, immune responses are reflected in neuroendocrine changes. This bidirectional communication is possible as both systems share receptors for common ligands and their coupled signaling pathways. The bidirectional information, which flows between the neuroendocrine and immune systems, functions to maintain and protect the internal homeostasis of the organism.

Hormones and neuropeptides, that provide the connection between the endocrine, the central nervous and the immune systems constitute specific axes, including the well studied hypothalamic–pituitary–adrenal (HPA) axis and the hypothalamic–pituitary–thyroid (HPT) axis, among others. Additionally, the autonomic nervous system also communicates with the lymphoid compartment through the release of norepinephrine and acetylcholine from sympathetic and parasympathetic nerves respectively [4]. Thus, it appears that multidirectional communication networks exist within the body that allow the signal transmission between these various systems during times of stress, injury, disease, infection, or other physiopathological situations.

In this manuscript the immunomodulatory action of thyroid hormones, their interrelationship with hormones of the HPA axis during stress conditions and their involvement in the stress-mediated immunosuppression that increases tumor growth will be discussed.

IMMUNOMODULATORY ROLE OF THYROID HORMONES

Although the participation of thyroid hormones in primary and secondary lymphopoiesis has been described [for review see 5], the effects of thyroid hormones on cells of the immune system received relatively less attention than those from hormones of the HPA axis [6,7], probably because of the dominating role of autoimmunity in the pathogenesis of thyroid disorders.

Interactions between HPT hormones and the immune system are mainly based on the existence of receptors for thyreotropic and thyroid hormones on lymphocytes or on the frequent immune alterations associated with physiological or

*Address correspondence to this author at the CEFYBO-CONICET-UBA, Paraguay 2155, piso 15 (Sector Uriburu), 1121ABG - Ciudad Autónoma de Buenos Aires, Argentina; Fax: 54 - 11 - 4508 - 3680, Ext. 106;
E-mail: grace@ffyb.uba.ar

1573-3955/10 $55.00+.00

pathological fluctuations of thyroid hormones. Also, cells of the immune system contain (produce, transport, concentrate or store) hormones. Thus, the presence of triiodothyronine (T3) was demonstrated in lymphocytes, mast cells and monocyte-macrophage-granulocytes from rat peritoneal fluid and blood, as well as in thymic lymphocytes [8]. This T3, that would be assumed to be an extrathyroidal source of the hormones as it was demonstrated *in vitro* that TSH treatment can elevate the T3 content in isolated immune cells [9], is probably necessary for maintaining cell proliferation and normal status in the immune system [8, 9].

Hypothyroidism in humans or experimentally induced hypothyroidism (i.e. induced by antithyroid agents) or thyroidectomy in rodents has shown to diminish thymic activity, while treatment with thyroid hormones reversed these effects [5, 10]. In addition, hypothyroid experimental conditions lead to spleen and lymph node involution, as well as to depressed humoral and cell-mediated immune responses [11-13]. Restoration of thyroid function, after thiourea-induced blockade, recomposed the humoral response to sheep red blood cells (SRBCs) in birds [14]. Results from animal models of hypothyroidism induced by antithyroid agents could lead to the confusion that the negative immunorregulatory effects observed are due to the agent. This is because it was shown that in vivo treatment with the antithyroid drug thiamazole inhibit the content of T3 in different immune cells, namely thymic lymphocytes, blood and peritoneal lymphocytes, monocytes, granulocytes and mast cells [15]. However, the reversion of thyrostatic drug effects on immune responses by T3 administration recall the attention to the fact that the lack of thyroid hormone is directly responsible for the immunomodulatory actions observed in these animals.

Moreover, suppression of cell-mediated immunity in severe human hypothyroidism, with improvement of lymphocyte function during a gradual return to the euthyroid state, has also been revealed [16]. Also, an important deficiency of IgA and IgM serum levels that correlate with lowfree thyroxine (T4) and with the severity of the pathology was described in children with congenital hypothyroidism [17].

Conversely, contradictory results exist in the literature on the effect of experimental hyperthyroidism on the humoral and cellular immunity. Thus, either a suppressing [18] or enhancing [13, 19, 20] effect of primary antibody responses; or of T and B lymphocyte proliferation [11, 13, 21] was demonstrated in hyperthyroid conditions. Many of these conditions in humans are autoimmune in nature and mainly related to antibodies against the TSH receptor that mimic the function of TSH, and cause disease by stimulating thyroid cells upon binding to the receptor [for review see 22]. So, enhancement or inhibition of humoral immune response by thyroid hormones thus can contribute to the pathological process by aggravating or suppressing the autoimmune state. The direction of change in the humoral immune response in the hyperthyroid state can elucidate the effect of thyroid hormones on humoral immunity.

Opposing data would reflect differences among species or among the hormonal treatment (doses, duration, type of hormone) established. Recently an integrative study of the HTP axis function and the immune status was performed in mice showing that lymphocytes from hyperthyroid mice displayed higher T and B-cell mitogen-induced proliferation compared with euthyroid animals, effect that was related to an increase in intracellular signals, i.e. protein kinase C (PKC), implicated in lymphocyte activation [21]. Also an increment in the release of crucial cytokines involved in T lymphocyte activation, namely interleukin-2 (IL-2) and interferon-γ (IFN-γ) by specific antigen-stimulation, as well as of IL-6 and tumor necrosis factor-α (TNF-α) by an inflammatory stimulus were observed under hyperthyroid conditions [21].

Most studies in humans that analyze the relationship between thyroid hormones and immune function were restricted to clinical populations with thyroid function disorders, mainly with autoimmune diseases [23-26]. These studies demonstrate that altered thyroid hormone concentration is associated with altered immunity, but whether these observations are directly due to the actions of thyroid hormones or underlying autoimmunity can not be ruled out. Results from animal models of hypo- and hyperthyroidism without autoimmune condition, not only show the importance of thyroid hormone circulating levels in immune modulation, but also that thyreotropic hormone levels are not responsible for the observed changes in immune function [21]. Additionally, recent data from our laboratory on transgenic mice overexpressing TRH gene and with higher levels of hypothalamic TRH than control animals, show an increment in lymphocyte activity and function, contrary to what was observed in hypothyroid mice that also have increased levels of TRH as well (unpublished observations).

Although it is difficult to elucidate the effect of thyroid hormones on the immune response in healthy euthyroid state because intricate interactions and modulations of many hormones and factors are involved in its regulation, a recent study in healthy elderly subjects, devoid of thyroid illness, demonstrates that higher concentrations of thyroid hormones, within normal physiological ranges, enhance innate and adaptive immunity through maintenance of specific cell populations and greater responsiveness to immune stimuli [27]. Also, it was demonstrated in mice that changes in thyroid hormone secretion related to ageing may be involved in the age-related immune dysfunction, as T4 treatment to aged mice was able to restore the age related decline of the immune efficiency [28].

In conclusion, these findings highlight the relation between thyroid function and immunity in healthy older individuals and the importance of these interactions under physiological conditions. Additionally, thyroid hormones can directly affect T lymphocyte proliferation [29] or dendritic cell maturation and function [30]. Moreover, it was recently demonstrated that the thyroid hormone receptor β1 and its coupled intracellular nongenomic signals play a role in modulating dendritic cell physiology and the intracellular mechanisms underlying these immunoregulatory effects [31].

All these data reinforce the HPT axis and immune system interaction, pointing to an enhancing role of thyroid hormones on immune function.

STRESS, IMMUNITY AND THYROID HORMONES

Some evidence arising from analysis of lymphocyte development and function in mice with genetic defects in the expression of thyroid hormones or their receptors suggested that these hormones are involved in maintaining immune system homeostasis in response to environmental changes or stress-mediated immunosuppression [6, 32-34].

With respect to stress, many studies have suggested that stress has a profound effect on immune function in both animals and humans [35]. Stress occurs when homeostasis is altered or is perceived to be threatened. In this situation re-establishment of homeostasis is achieved by adaptive responses. Neuroendocrine hormones participate in both the regulation and the restoration of homeostasis and are involved in the pathogenesis of diseases characterized by dyshomeostasis or cacostasis [36]. Despite that acute stress is related with the activation of acute phase immune responses which are critical for rapid and effective pathogen clearance upon infection [37, 38], chronic stress has been associated to diminished immunity. In fact it is considered a key factor in the development of several pathologies, including psychiatric diseases, disruption of neuroendocrine systems, alterations of the immune response and even cancer [35, 39]. This is evidenced by a reduction of T cytotoxic and natural killer (NK) activities [40], by a decrease in T-lymphocyte proliferative response to mitogens through a reduction of the intracellular signals involved in T cell activation [41-44], impairment of antibody production [42, 43] and changes in cytokine secretion [45].

A variety of potential mechanisms by which stressors can alter immune function were proposed. One of these mechanisms would include the alteration of the hypothalamus-pituitary axis and the corresponding target endocrine glands (namely thyroid, gonads and adrenal) that in turn modulate the immune function [46, 47]. The most common mechanism studied is the stress-mediated activation of both the HPA axis and the autonomic nervous system, leading to altered levels of the inmunomodulators ACTH and corticosterone by one hand or cathecolamines by the other and thus affecting immune responses [48, 49]. Chronic hyper-activation of the hypothalamus-pituitary axis is associated with the suppression of reproductive, growth, thyroid and immune functions that may lead to various pathological states [50, 51]. In this context, several works have indicated that stress can alter thyroid hormone secretion, as corticosterone, the end product of HPA axis activation, has been suggested to play a role in HPT axis regulation. Indeed, in a stress model in rats, obtained by repeated exposure to mild-electric foot-shock, a decrease in peripheral thyroid hormone levels was found [52]. Also, it has been proposed that chronic exercise stress induces a hormonal status similar to that of euthyroid sick syndrome (ESS), with suppression of T3 and elevation of rT3 plasma levels. This effect is probably related to cathecolamine-mediated activation of NF-kappaB that in turns inactivates T3-dependent 5'-deiodinase gene expression and enzyme activity, leading to the generation of ESS in the periphery [53].

Differences in adaptative responses to stress would be related to either activation or suppression of the thyroid system. It was described that traumatic stress is accompanied by activation of thyroid function, as it was reported that patients with hyperthyroidism account a history of more stressful life events than do members of a control population [54-56]. Conversely, in chronic posttraumatic stress disorder patients significantly lower plasma cortisol and TSH levels were found respect to control subjects [57] and also chronic stress has been generally associated with suppression of thyroid axis function [58]. Furthermore, chronic stress has been demonstrated to be the major cause leading to depressive disorders [59,60] and accordingly, patients with melancholic depression, as well as anorectics and highly trained athletes have significantly lower thyroid hormone concentrations than controls [61-63].

In addition to these observations the relationship among stress, thyroid axis and immunity has been recently demonstrated in a murine model of chronic stress. A reduction in serum levels of thyroid hormones, mainly of the biologically active T3 was found in stressed animals that correlated with lower titers of specific antibodies after alloimmunization [64]. Thyroidal inhibition following submission to diverse stressors was also described in other species [65]. Furthermore, substitutive treatment with T4 in stressed animals not only restored thyroid hormone serum levels, but significantly increased alloantibody production, thus suggesting that chronic stress induces an alteration of thyroid axis function that in turn alters the immune response [64]. During stress, a suppressed secretion of TSH and a decreased conversion of the relatively inactive T4 to the potent biologically active T3 in peripheral tissues has been described [66], so a major decrease in T3 levels would be responsible of the immune response alterations. Similarly, in stressed animals reduced lymphocyte reactivity in response to mitogens correlates with decreased serum thyroid hormone levels that were precede by an increase in corticosterone levels [67]. Substitutive T4 treatment in stressed animals improved significantly proliferative T-cell responses [67]. Taken together these results suggest that chronic stress conditions by activating HPA axis lead to the alteration of thyroid function that in turn affects T-cell response. Despite their demonstrated direct action on immune cells [29, 30], the possibility that thyroid hormones modulation of immune responses would be mediated by their regulation of other endocrine mediators can not be ruled out. In fact, it has been described that both T3 and T4 in turn influence the levels of plasma and adrenal corticosterone [68-70] and it is well known the modulatory action of glucocorticoids on the immune function [71].

On the other hand, environmental changes as cold or heat stress can also alter thyroid function leading to immune regulation. During cold stress there is an increase in the HPT axis function [32]. In fact, timing and magnitude of cold adaptation depend, to some extent, on thyroid function, being high T3 concentrations important for energy expenditure and dissipation of heat in special tissues [72]. Increased thyroid hormone secretion during cold stress would then exert immunoenhancing effects. An interrelation between HPA and HPT axis was also described to take place during cold stress. Thus, Fukuhara *et al*. [73] have reported that acute exposure to cold in rats transiently increased plasma ACTH, and corticosterone, which tended to decrease with continued cold exposure. Thyroid hormones on the

other hand were elevated after acute but remained elevated after continue cold exposure. Therefore, it is reasonable to speculate that increased secretion of thyroid hormones during cold stress serves, in addition to their well known actions on regulating basal metabolic rate, to counter the immunosuppressive actions of the acutely increased glucocorticoid secretion and to further maintain immune system homeostasis during continued cold exposure.

Despite the picture is still incomplete, Fig. (**1**) would help to elucidate the neurochemical and hormonal mechanisms through which stress is able to alter immunity as it shows how endocrine mechanisms would tune immune system function during acute and the chronic stress. In this way (panel **A**), a stressful situation would first activate HPA axis leading to an increased production of glucocorticoids, that would negatively affect the immune system, but in turn activation of HPT axis (directly or as a consequence of HPA axis activity) would led to the increment in thyroid hormone levels. This last one may not only counteract those immunosuppressive effects, but tend to normalize glucocorticoids levels as observed during chronic submission to stress (panel **B**). In this case, the persistence of stressors would shut down HPT axis leading to low levels of thyroid hormones with the consequently negative regulation of immune system components. These findings on endocrine system modulation of the immune system during stress would participate in the etiology of many diseases including cancer.

THYROID HORMONES AND STRESS IN TUMOR SURVEILLANCE

Among stress-mediated alterations of immunity, reduction of T cytotoxic and NK activities among others were described. Both T cells and NK cells are the major components of anti-tumoral immunity. The CD8+ T-cytotoxic and CD4+ T-helper lymphocytes are crucial regulators of tumor growth [74, 75]. NK cells are potent effectors against tumor cells by inducing cytotoxicity [76, 77]. Cellular cytokines, such as TNF-α and IFN-γ are key mediators in these processes [78, 79]. So, chronic stress could be related to a worse outcome in cancer disease by suppressing anti-tumoral immunity [80-82]. Despite that in humans the role of stress as a risk factor for cancer development has not been conclusively stated for some tumor pathologies [83, 84], over the past 25 years, epidemiological and clinical studies have linked psychological factors such as stress, chronic depression, and lack of social support to the incidence and progression of cancer [85]. Thus, in an animal model of sarcoma in which tumor development is only achieved if rats are previously treated with the immunosuppressive agent cyclosporine (CS), Basso *et al.* [86] demonstrated that exposure of animals to a chronic variable stress procedure after tumor inoculation, facilitated tumor growth even in rats without CS administration. Stress-mediated increment in the development and progression of tumors was demonstrated in

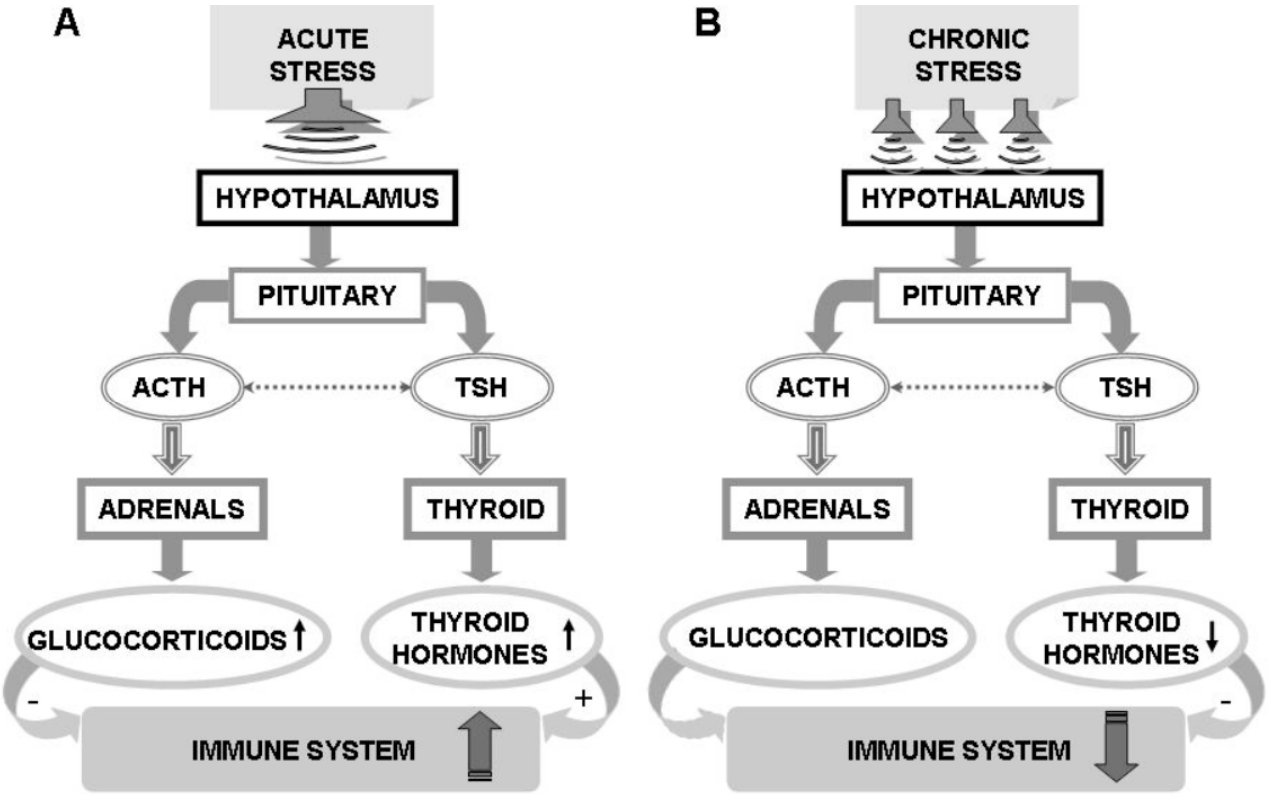

Fig. (1). Hormonal mechanisms participating in acute (Panel **A**) or chronic (Panel **B**) stress modulation of immunity. Acute or chronic stress lead to a differential alteration of the hypothalamus-pituitary axis, modifying circulating levels of glucocorticoids and thyroid hormones, both involved in modulating the immune response. Acute stress increases both the immunosuppressive glucocorticoid and the compensatory immunostimulating thyroid hormone levels. The final consequence of this is the activation of immune responses. On the contrary, chronic stress induces the hyper-activation of the hypothalamus-pituitary axis that suppresses the HPT axis function and reduces the levels of thyroid hormones thus negatively regulating the immune response.

different types of cancer animal models and although the mechanisms underlying these observations are not completely understood, recent studies have begun to unravel some of the immune components that are modulated by the neuroendocrine effects on tumor growth and metastasis [85].

In this sense, Frick *et al*. [87] have demonstrated in a syngeneic murine lymphoma model that, the T-cell response, but not the NK activity, is negatively affected in mice submitted to chronic restraint stress which additionally display an enhancement of tumor growth (i.e. increased tumor size and proliferation and reduced animal survival). About the effects of stress on cognate immunity a reduction of T-, but not B-cell proliferation and of lymph node CD4+, but not CD8+, T-lymphocyte subsets were described, suggesting that stress mainly affects T-helper immunity [87]. Similarly, Saul *et al*. [88], also found a reduction of tumor infiltrating CD4+, but not CD8+- T cells in restraint stressed mice bearing squamous cell carcinoma tumors. However, Stefanski and Engler [89] found a reduction of both T-cell subsets in blood from socially stressed rats and Silberman *et al.* [67] found no differences at all between CD4+ and CD8+ T-lymphocytes in lymph nodes from mice exposed to chronic mild stress. Differences between chronic mild and restraint models could be related to the type of stressor applied in each case. Thus in the heterotrophic chronic mild stress model different stressors of moderate intensity are randomly applied, while in the homotrophic chronic restraint and social stress models only one type of strong stressor is involved. By other hand, it was also demonstrated by other authors that NK activity can remain unaltered after stress exposure depending on the stressor applied and its duration [90]. In particular, it was shown that chronic restraint stress did not modify NK activity in rats [91]. Although the participation of NK cells in these processes can not be ruled out, NK activity has classically been related to the inhibition of metastasis [92] rather than to the control of tumor cell proliferation, mainly mediated by T cells [93].

Additionally, stressed mice had a reduced specific cytotoxic response against these tumor cells and reduced levels of TNF-α and IFN-γ cytokines, thus strengthening that chronic exposure to stress promotes cancer establishment and subsequent progression, probably by depressing T-cell mediated immunity. Similarly, Saul *et al.* [88] demonstrated that chronic stress increases the susceptibility to UV-induced squamous cell carcinoma in a mouse model by suppressing type 1 cytokines and protective T cells and increasing regulatory/suppressor T cell numbers. Furthermore, stress hormones have been reported to inhibit systemically TNF-α and IFN-γ [81]. Several findings indicate that different types of stressful conditions results in the promotion of distinct murine tumors, namely ovarian carcinoma growth and angiogenesis [94], B16F10 melanoma [95] and Ehrlich tumor growth [96].

In addition, Freire-Garabal *et al*. [97] found that chronic auditory stress results in a modification of the incidence of breast cancer in mice and development of lung metastases in rats and that nefazodone, a serotonin and norepinephrine reuptake inhibitor and a serotonin receptor antagonist antidepressant, reduce the effects of stress in tumor development if both breast and lung tumors. Similarly, Frick *et al.* [98] found that fluoxetine, a selective serotonin reuptake inhibitor, inhibits tumor growth through the modulation of T-cell-mediated immunity.

Although other mechanisms may be involved, the T-cell immunity impairment as well as the tumor progression enhancement emphasizes the importance of the therapeutic management of stress to improve the prognosis of cancer patients [87]. Among the possible mediators involved in stress-mediated immunosuppression leading to the facilitation of tumor growth the participation of the neuroendocrine axes HPA and HPT and the autonomic nervous system (ANS) in the experimental tumor model of murine lymphoma was recently studied [99]. Classic stress-related hormones glucocorticoids and catecholamines were increased by a single session of restraint stress, but chronic exposure to the stressor normalized their levels as it was demonstrated for the chronic mild stress model [67]. In contrast, thyroid hormones were down regulated only after chronic restraint stress as shown in other models of stress [52, 53, 64, 67]. This decrease in thyroid hormone levels that is accompanied by impairment in T-cell reactivity was also observed in chronically stressed mice bearing solid tumors. At the molecular level intracellular signals involving the translocation to cell membranes of specific protein kinase C (PKC) isoforms θ and α, known to be involved in T-cell activation, was also found [99]. Thyroxin replacement in these animals, which restored the euthyroid status, not only reversed the effect on T lymphocyte function and signaling events, but also prevented the exacerbation of tumor growth and the inhibition of the specific cytotoxic response against lymphoma cells, as well as of cytokine expression. It has been shown that PKC plays an important role in CD8+ cytotoxic T lymphocyte effectors responses during tumor rejection [100]. In addition, PKC-θ also modulates IL-2 transcription in T-lymphocytes [101]. Knockout mice for PKC-θ exhibit reduced T-cell proliferation and IL-2 production, whereas knock-in mice for PKC-α display the opposite phenotype [102]. So, PKC (isoforms θ and α) might be one of the major intracellular signalers involved in the actions of thyroid hormones in antitumor immunity after chronic stress, a process that in turn alters tumor prognosis. The relevance of thyroid hormone participation in the stress-mediated enhancement of the lymphoma model described is strengthen by the fact that in the particular case of leukemias/lymphomas, both glucocorticoids and cathecolamines often induce apoptosis in tumor cells rather than proliferation [103-105]. In fact, Frick *et al.* [99] demonstrated through *in vitro* assays that dexamethasone and epinephrine blocked lymphoma cell proliferation and although no direct action of thyroid hormones was found on this lymphoma cell, direct stimulation of proliferation was described to be induced by both T3 and T4 in another T lymphoma cell line [29].

These findings, which are summarized in Fig (**2**), point to the relevance of HPT deregulation in response to the stressor and also indicate a potential therapeutic action of thyroxin in the adjuvant treatment of stress-related disorders such as immunosuppression and cancer.

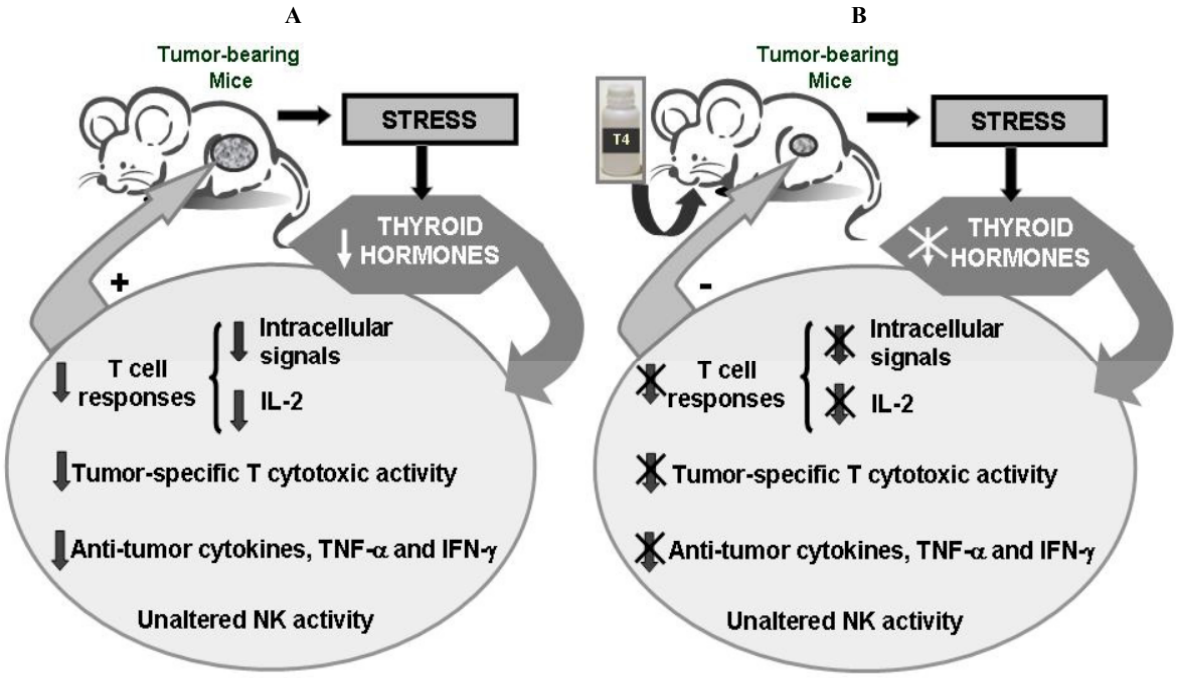

Fig. (2). Mechanisms involved in stress-mediated alteration of HPT axis leading to anti-tumor immunosuppression in tumor bearing mice (Panel **A**). Restoration of both thyroid hormone levels and immune function by thyroxine administration (Panel **B**).

CONCLUSION

The immune-neuroendocrine interactions are involved in numerous physiological and pathophysiological conditions. This crosstalk is important to homeostasis, since interactions can produce various appropriate adaptative responses when homeostasis is threatened. The interactions with the HPA axis by increasing the production of glucocorticoids may represent a mechanism to control the overshooting immune system-mediated inflammatory response. The HPT axis counteracting these immunosuppressive actions would balance the scenario to keep the homeostatic function of the immune system.

Stress has long been suspected to play a role in the etiology of many diseases and may be detrimental to health. Nowadays, the communication between the neuroendocrine and the immune systems is well established and there is sufficient evidence indicating that the magnitude of stress-associated immune deregulation is large enough to have health implications. In stress conditions, an immunosuppressive state, in part mediated through a diminished production of thyroid hormones, lead to an increase susceptibility to infections and cancer. Restoration of thyroid function in stressed tumor-bearing subjects will improve tumor management by increasing anti-tumor immune responses.

The mechanistic understanding of these interrelationships is important for establishing new pharmacological approaches for improving the treatment of cancer patient. Precisely these findings indicate a potential therapeutic action of thyroxin in the adjuvant treatment of stress-related disorders such as immunosuppression and cancer. In fact, administration of thyroid hormones as adjuvant for the treatment of AIDS [106] and tumors [107] were recently proposed. Finally, these evidences strengthen the important role that thyroid hormone play in immunomodulation, with a special emphasis in their participation as neuroendocrine regulators of stress-mediated immune deficit.

ACKNOWLEDGEMENTS

Financial contributions to the work being reported are grants from the National Research Council (PIP CONICET N° 6049 and 6054) and from the University of Buenos Aires (UBACYT B041 and B039).

REFERENCES

[1] Blalock JE. A molecular basis for bidirectional communication between the immune and neuroendocrine systems. Physiol Rev 1989; 69(1): 1–32.
[2] Melmed S. The immuno-neuroendocrine interface. J Clin Invest 2001; 108(11): 1563–6.
[3] Wrona D. Neural-immune interactions: an integrative view of the bidirectional relationship between the brain and immune systems. J Neuroimmunol 2006; 172(1-2): 38-58.
[4] Taub DD. Neuroendocrine interactions in the immune system. Cell Immunol 2008; 252(1-2): 1-6.
[5] Fabris N, Mocchegiani E, Provinciali M. Pituitary thyroid axis and immune system: a reciprocal neuroendocrine immune interaction. Horm Res 1995; 43(1-3): 29–38.
[6] Dorshkind K, Horseman ND. Anterior pituitary hormones, stress, and immune system homeostasis. Bioessays 2001; 23(3): 288–94.
[7] Wang H-C, Klein JR. Immune function of thyroid stimulating hormone and receptor. Crit Rev Immunol 2001; 21(4): 323–37.
[8] Csaba G, Kovács P, Pállinger É. Immunologically demonstrable hormones and hormone-like molecules in rat white blood cells and mast cells. Cell Biol Internat 2004; 28(6): 487-90.
[9] Csaba G, Pállinger E. Thyrotropic hormone (TSH) regulation of triiodothyronine (T3) concentration in immune cells. Inflamm Res 2009; 58(3):151-4.
[10] Ribeiro-Carvalho MM, Lima-Quaresma KR, Mouço T, Carvalho VF, Mello-Coelho V, Savino W. Triiodothyronine modulates thymocyte migration. Scand J Immunol 2007; 66(1): 17-25.
[11] Chatterjee S, Chandel AS. Immunomodulatory role of thyroid hormones: in vivo effect of thyroid hormones on the blastogenic

response to lymphoid tissue. Acta Endocrinologica 1983; 103(1): 95–100.

[12] Ohashi H, Itoh M. Effects of thyroid hormones on lymphocyte phenotypes in rats: changes in lymphocyte subsets related to thyroid function. Endocr Regul 1994; 28(3): 117–23.

[13] Klecha AJ, Genaro AM, Lysionek AE, Caro RA, Coluccia GA, Cremaschi GA. Experimental evidence pointing to the bidirectional interaction between the immune system and the thyroid axis. Int J Immunopharmacol 2000; 22(7): 491–500.

[14] Keast D, Ayre DJ. Antibody regulation in birds by thyroid hormone. Dev Comp Immunol 1980; 4(2): 323–30.

[15] Csaba G, Kovács P, Pállinger É. Effect of the inhibition of triiodothyronine (T3) production by thiamazole on the T3 and serotonin content of immune cells. Life Sci 2005; 76(18): 2043-52.

[16] Schoenfeld PS, Myers JW, Myers L, LaRocque JC. Suppression of cell-mediated immunity in hypothyroidism. South Med J 1995; 88(3): 347–9.

[17] Stagi S, Azzari C, Bindi G, *et al*. Undetectable serum IgA and low IgM concentration in children with congenital hypothyroidism. Clin Immunol 2005; 116(1): 94-8.

[18] Bittencourt CS, Azzolini AECS, Ferreira DA, Assis-Pandochi AI. Antibody responses in hyperthyroid rats. Int Immunopharmacol 2007; 7(7): 989-93.

[19] Vinayagamoorthi R, Koner BC, Kavitha S, Nandakumar DN, Padma Priya P, Goswami K. Potentiation of humoral immune response and activation of NF-kappaB pathway in lymphocytes in experimentally induced hyperthyroid rats. Cell Immunol 2005; 238(1): 56-60.

[20] Nandakumar DN, Koner BC, Vinayagamoorthi R, *et al*. Activation of NF-κB in lymphocytes and increase in serum immunoglobulin in hyperthyroidism: Possible role of oxidative stress. Immunobiology 2008; 213(5): 409-15.

[21] Klecha AJ, Genaro AM, Gorelik G, *et al*. Integrative study of hypothalamus- pituitary-thyroid-immune system interaction: thyroid hormone mediated modulation of lymphocyte activity through the protein kinase C signaling pathway. J Endocrinol 2006; 189(1): 45–55.

[22] Klecha AJ, Barreiro Arcos ML, Frick L, Genaro AM, Cremaschi G. Immune-endocrine interactions in autoimmune thyroid diseases. Neuroimmunomodulation 2008; 15(1): 68–75.

[23] Nakanishi K, Taniguchi Y, Ohta Y. Increased soluble interleukin 2 receptor levels in autoimmune thyroid disease. Acta Endocrinol 1991; 125(3): 253–8.

[24] Covas MI, Esquerda A, Garcia-Rico A, Mahy N. Peripheral blood T-lymphocyte subsets in autoimmune thyroid disease. J Investig Allergol Clin Immunol 1992; 2(3): 131–5.

[25] Mariotti S, Caturegli P, Barbesino G, *et al*. Thyroid function and thyroid autoimmunity independently modulate serum concentration of soluble interleukin 2 (IL-2) receptor (sIL-2R) in thyroid diseases. Clin Endocrinol 1992; 37(5): 415–22.

[26] Bossowski A, Urban M, Stasiak-Barmuta A. Analysis of changes in the percentage of B (CD19) and T (CD3) lymphocytes, subsets CD4, CD8 and their memory (CD45RO), and naive (CD45RA) T cells in children with immune and non-immune thyroid diseases. J Pediatr Endocrinol Metab 2003; 16(1): 63–70.

[27] Hodkinson CF, Simpson EEA, Beattie JH, *et al*. Preliminary evidence of immune function modulation by thyroid hormones in healthy men and women aged 55–70 years. J Endocrinol 2009; 202(1): 55–63.

[28] El-Shaikh KA, Gabry MS, Othman GA. Recovery of age-dependent immunological deterioration in old mice by thyroxine treatment. J Anim Physiol Anim Nutr (Berl) 2006; 90(5-6): 244-54.

[29] Barreiro Arcos ML, Gorelik GJ, Klecha AJ, Genaro AM, Cremaschi GA. Thyroid hormones increase inducible nitric oxide synthase gene expression down-stream PKC ζ in tumor murine T lymphocytes. Am J Physiol Cell Physiol 2006; 291(2): C327-36.

[30] Mascanfroni I, Montesinos MM, Susperreguy S, *et al*. Control of dendritic cell maturation and function by triiodothyronine. FASEB J 2008; 22(4): 1032-42.

[31] Mascanfroni ID, Montesinos MD, Alamino VA, *et al*. Nuclear factor NF-κB-dependent thyroid hormone receptor β1-expression controls dendritic cell function *via* AKT signaling. J Biol Chem 2010; 285(13): 9569-82.

[32] Davis SL. Environmental modulation of the immune system *via* the endocrine system. Domest Anim Endocrinol 1998; 15(5): 283–9.

[33] Dorshkind K, Horseman ND. The roles of prolactin, growth hormone, insulin-like growth factor-I, and thyroid hormones in lymphocyte development and function: insights from genetic models of hormone and hormone receptor deficiency. Endocrine Rev 2000; 21(3): 292–312.

[34] Foster MP, Jensen ER, Montecino-Rodriguez E, Leathers H, Horseman N, Dorshkind K. Humoral and cell-mediated immunity in mice with genetic deficiencies of prolactin, growth hormone, insulin-like growth factor-I, and thyroid hormone. Clin Immunol. 2000; 96(2), 140–9.

[35] Glaser R, Kiecolt-Glaser JK. Stress-induced immune dysfunction. Implications for health. Nat Rev Immunol 2005; 5(3): 243–51.

[36] Chrousos GP. Stress and disorders of the stress system. Nat Rev Endocrinol 2009; 5(7): 374-81.

[37] Ali H, Haribabu B, Richardson R, Snyderman R. Mechanisms of inflammation and leukocyte activation. Med Clin North Am 1997; 81(1): 1–28.

[38] Tilg H, Dinarello CA, Mier JW. IL-6 and APPs: anti-inflammatory and immunosuppressive mediators. Immunol Today 1997; 18(9): 428–32.

[39] Reiche EM, Nunes SO, Morimoto HK. Stress, depression, the immune system, and cancer. Lancet Oncol 2004; 5(10): 617–25.

[40] Nuñez MJ, Balboa J, Rodrigo E, Breñilla J, Gonzalez-Peteiro M, Freire-Garabal M. Effects of fluoxetine on cellular immune response in stressed mice. Neurosci Lett 2006; 396(3): 247–51.

[41] Edgar VA, Silberman DM, Cremaschi GA, Zieher LM, Genaro AM. Altered lymphocyte catecholamine reactivity in mice subjected to chronic mild stress. Biochem Pharmacol 2003; 65(1):15–23.

[42] Silberman DM, Wald M, Genaro AM. Acute and chronic stress exert opposing effects on antibody responses associated with changes in stress hormone regulation of T-lymphocyte reactivity. J. Neuroimmunol 2003; 144(1-2): 53–60.

[43] Silberman DM, Ayelli-Edgar V, Zorrilla-Zubilete M, Zieher LM, Genaro AM. Impaired T-cell dependent humoral response and its relationship with T lymphocyte sensitivity to stress hormones in a chronic mild stress model of depression. Brain Behav Immun 2004; 18(1): 81–90.

[44] Silberman DM, Zorrilla-Zubilete M, Cremaschi GA, Genaro AM. Protein kinase C-dependent NF-kB activation is altered in T cells by chronic stress. Cell Mol Life Sci 2005; 62(15): 1744-54.

[45] Merlot E, Moze E, Dantzer R, Neveu PJ. Cytokine production by spleen cells after social defeat in mice: Activation of T cells and reduced inhibition by glucocorticoids. Stress 2004; 7(1): 55–61.

[46] Besedovsky HO, del Rey A. Feed-back interactions between immunological cells and the hypothalamus-pituitary-adrenal axis. Neth J Med 1991; 39(3-4): 274-80.

[47] Savino W, Dardenne M. Immune-neuroendocrine interactions. Immunol Today 1995; 16(7): 318-22.

[48] Geenen R, Van Middendorp H, Bijlsma JW. The impact of stressors on health status and hypothalamic-pituitary-adrenal axis and autonomic nervous system responsiveness in rheumatoid arthritis. Ann N Y Acad Sci 2006; 1069: 77-97.

[49] Chen E, Miller GE. Stress and inflammation in exacerbations of asthma. Brain Behav Immun 2007; 21(8): 993-9.

[50] Charmandari E, Kino T, Souvatzoglou E, Chrousos GP. Pediatric stress: hormonal mediators and human development. Horm Res 2003; 59(4): 161-79.

[51] Maccari S, Morley-Fletcher S. Effects of prenatal restraint stress on the hypothalamus-pituitary-adrenal axis and related behavioural and neurobiological alterations. Psychoneuroendocrinology 2007; 32: S10-5.

[52] Helmreich DL, Parfitt DB, Lu XY, Akil H, Watson SJ. Relation between the hypothalamic-pituitary-thyroid (HPT) axis and the hypothalamic-pituitary-adrenal (HPA) axis during repeated stress. Neuroendocrinology 2005; 81(3): 183-92.

[53] Mastorakos G, Pavlatou M. Exercise as a stress model and the interplay between the hypothalamus-pituitary-adrenal and the hypothalamus-pituitary-thyroid axes. Horm Metab Res 2005; 37(9): 577-84.

[54] Winsa B, Adami HO, Bergström R, *et al*. Stressful life events and Graves' disease. Lancet 1991; 338(8781): 1475-9.

[55] Radosavljević VR, Janković SM, Marinković JM. Stressful life events in the pathogenesis of Graves' disease. Eur J Endocrinol 1996; 134(6): 699-701.

[56] Wang S, Mason J. Elevations of serum T3 levels and their association with symptoms in World War II veterans with combat-related posttraumatic stress disorder: replication of findings in Vietnam combat veterans. Psychosom Med 1999; 61(2): 131-8.

[57] Olff M, Güzelcan Y, de Vries GJ, Assies J, Gersons BP. HPA- and HPT-axis alterations in chronic posttraumatic stress disorder. Psychoneuroendocrinology 2006; 31(10): 1220-30.

[58] Chrousos GP, Gold PW. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. JAMA 1992; 267(9): 1244-52.

[59] Anisman H, Zacharko RM. Depression as a consequence of inadequate neurochemical adaptation in response to stressors. Br J Psychiatry 1992; (15): 36-43.

[60] Pittenger C, Duman RS. Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology 2008; 33(1): 88-109.

[61] Stipcević T, Pivac N, Kozarić-Kovacić D, Mück-Seler D. Thyroid activity in patients with major depression. Coll Antropol 2008; 32(3): 973-6.

[62] Usdan L, Khaodhiar S, Apovian L. The endocrinopathies of anorexia nervosa. Endocr Pract 2008; 14(8): 1055-63.

[63] Kanaka-Gantenbein C. The impact of exercise on thyroid hormone metabolism in children and adolescents. Horm Metab Res 2005; 37 (9) :563-5.

[64] Cremaschi GA, Gorelik G, Klecha A, Lysionek AE, Genaro AM. Chronic stress influences the immune system through the thyroid axis. Life Sci 2000; 67(26): 3171–9.

[65] Ray PP, Sengupta A, Chaudhuri-Sengupta S, Maiti BR. Thyroidal inhibition following diverse stress in soft-shelled turtle, Lissemys punctata punctata bonnoterre. Acta Biol Hung 2008; 59(4): 403-12.

[66] Stratakis CA, Chrousos GP. Neuroendocrinology and pathophysiology of the stress system. Ann NY Acad Sci 1995; 771: 1–18.

[67] Silberman DM, Wald M, Genaro AM. Effects of chronic mild stress on lymphocyte proliferative response. Participation of serum thyroid hormones and corticosterone. Int Immunopharmacol 2002; 2(4): 487-97.

[68] Lo MJ, Kau MM, Chen YH, *et al*. Acute effects of thyroid hormones on the production of adrenal cAMP and corticosterone in male rats. Am J Physiol 1998; 274(2Pt1): E238–45.

[69] Johnson EO, Kamilaris TC, Calogero AE, Gold PW, Chrousos GP. Experimentally-induced hyperthyroidism is associated with activation of the rat hypothalamic-pituitary-adrenal axis. Eur J Endocriol 2005; 153(1): 177-85.

[70] Tohei A. Studies on the functional relationship between thyroid, adrenal and gonadal hormones. J Reprod Dev 2004; 50(1): 9-20.

[71] Reichardt HM. Immunomodulatory activities of glucocorticoids: insights from transgenesis and gene targeting. Curr Pharm Des 2004; 10(23): 2797-805.

[72] Laurberg P, Andersen S, Karmisholt J. Cold adaptation and thyroid hormone metabolism. Horm Metab Res 2005; 37(9): 545-9.

[73] Fukuhara K, Kvetnansky R, Cizza G, *et al*. Interrelations between sympathoadrenal system and hypothalamo-pituitary-adrenocortical/ thyroid systems in rats exposed to cold stress. J Neuroendocrinol 1996; 8(7): 533-41.

[74] Foss FM. Immunologic mechanisms of antitumor activity. Semin Oncol 2002; 29(3Suppl7): 5–11.

[75] Ostrand-Rosenberg S. CD4+ T lymphocytes: A critical component of antitumor immunity. Cancer Invest 2005; 23(5): 413–9.

[76] Abbas AK, Lichtman AH, Pober JS. In: Cellular and Molecular Immunology. 4th ed. Philadelphia: McGraw-Hill 2000; 2pp. 35-69.

[77] O'Hanlon LH. Natural born killers: NK cells drafted into the cancer fight. J Natl Cancer Inst 2004; 96(9): 651–3.

[78] Knutson KL, Disis ML. Tumor antigen-specific T helper cells in cancer immunity and immunotherapy. Cancer Immunol Immunother 2005, 54(8): 721–8.

[79] Dredge K, Marriott JB, Todryk SM, Dalgleish AG. Adjuvants and the promotion of Th1-type cytokines in tumor immunotherapy. Cancer Immunol Immunother 2002; 51(10): 521–31.

[80] Godbout JP, Glaser R. Stress-induced immune dysregulation: implications for wound healing, infectious disease and cancer. J Neuroimmune Pharmacol 2006; 1(4): 421-7.

[81] Calcagni E, Elenkov I. Stress system activity, innate and T helper cytokines, and susceptibility to immune-related diseases. Ann N Y Acad Sci 2006; 1069: 62–76.

[82] Armaiz-Pena GN, Lutgendorf SK, Cole SW, Sood AK. Neuroendocrine modulation of cancer progression. Brain Behav Immun 2009; 23(1): 10-5.

[83] Aragona M, Muscatello MR, Losi E, *et al*. Lymphocyte number and stress parameter modifications in untreated breast cancer patients with depressive mood and previous life stress. J Exp Ther Oncol 1996; 1(6): 354-60.

[84] Butow PN, Hiller JE, Price MA, Thackway SV, Kricker A, Tennant CC. Epidemiological evidence for a relationship between life events, coping style, and personality factors in the development of breast cancer. J Psychosom Res 2000; 49(3): 169-81.

[85] Thaker PH, Sood AK. Neuroendocrine influences on cancer biology. Semin Cancer Biol 2008; 18(3): 164-70.

[86] Basso AM, Depiante-Depaoli M, Molina VA. Chronic variable stress facilitates tumoral growth: reversal by imipramine administration. Life Sci 1992; 50(23): 1789-96.

[87] Frick LR, Arcos ML, Rapanelli M, Zappia MP, Brocco M, Mongini C, Genaro AM, Cremaschi GA. Chronic restraint stress impairs T-cell immunity and promotes tumor progression in mice. Stress 2009; 12(2): 134-43.

[88] Saul AN, Oberyszyn TM, Daugherty C, *et al*. Chronic stress and susceptibility to skin cancer. J Natl Cancer Inst 2005; 97(23): 1760–7.

[89] Stefanski V, Engler H. Social stress, dominance and blood cellular immunity. J Neuroimmunol 1999; 94(1-2):144–52.

[90] Oishi K, Nishio N, Konishi K, *et al*. Differential effects of physical and psychological stressors on immune functions of rats. Stress 2003; 6(1): 33–40.

[91] Steplewski Z, Vogel WH. Total leukocytes, T-cell subpopulation and natural killer (NK) cell activity in rats exposed to restraint stress. Life Sci 1986; 38(26): 2419–27.

[92] Christianson SW, Greiner DL, Schweitzer IB, *et al*. Role of natural killer cells on engraftment of human lymphoid cells and on metastasis of human T-lymphoblastoid leukemia cells in C57BL/6J-scid mice and in C57BL/6J-scid bg mice. Cell Immunol 1996; 171(2): 186–99.

[93] Turner WJ, Chatten J, Lampson LA. Human neuroblastoma cell growth in xenogeneic hosts: Comparison of T cell-deficient and NK-deficient hosts, and subcutaneous or intravenous injection routes. J Neurooncol 1990; 8(2): 121–32.

[94] Thaker PH, Han LY, Kamat AA, *et al*. Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. Nat Med 2006; 12(8): 939-44.

[95] Sá-Rocha VM, Sá-Rocha LC, Palermo-Neto J. Variations in behavior, innate immunity and host resistance to B16F10 melanoma growth in mice that present social stable hierarchical ranks. Physiol Behav 2006; 88(1-2): 108-15.

[96] Morgulis MS, Stankevicius D, Sá-Rocha LC, Palermo-Neto J. Cohabitation with a sick cage mate: consequences on behavior and on ehrlich tumor growth. Neuroimmunomodulation 2004; 11(1): 49-57.

[97] Freire-Garabal M, Rey-Méndez M, García-Vallejo LA, *et al*. Effects of nefazodone on the development of experimentally induced tumors in stressed rodents. Psychopharmacology 2004; 176(3-4): 233–8.

[98] Frick LR, Palumbo ML, Zappia MP, Brocco MA, Cremaschi GA, Genaro AM. Inhibitory effect of fluoxetine on lymphoma growth through the modulation of antitumor T-cell response by serotonin-dependent and independent mechanisms. Biochem Pharmacol 2008; 75(9): 1817-26.

[99] Frick LR, Rapanelli M, Bussmann UA, *et al*. Involvement of thyroid hormones in the alterations of T-cell immunity and tumor progression induced by chronic stress. Biol Psychiatry 2009; 65(11): 935-42.

[100] Nesic D, Jhaver KG, Vukmanovic S. The role of protein kinase C in CD8+ T lymphocyte effector responses. J Immunol 1997; 159(2): 582–90.

[101] Sun Z, Arendt CW, Ellmeier W, *et al*. PKC-theta is required for TCR-induced NF-kappaB activation in mature but not immature T lymphocytes. Nature 2000; 404(6776): 402-7.

[102] Pfeifhofer C, Kofler K, Gruber T, *et al*. Protein kinase C θ affects Ca^{2+} mobilization and NFAT cell activation in primary mouse T-cells. J Exp Med 2003; 197(11): 1525–35.

[103] Yan L, Herrmann V, Hofer JK, Insel PA. β-adrenergic receptor/cAMP-mediated signaling and apoptosis of S49

lymphoma cells. Am J Physiol Cell Physiol 2000; 279(5): C1665–74.

[104] Lu J, Quearry B, Harada H. p38-MAP kinase activation followed by BIM induction is essential for glucocorticoid-induced apoptosis in lymphoblastic leukemia cells. FEBS Lett 2006; 580(14): 3539 – 44.

[105] Laane E, Panaretakis T, Pokrovskaja K, *et al*. Dexamethasone-induced apoptosis in acute lymphoblastic leukemia involves differential regulation of Bcl-2 family members. Haematologica 2007; 92(11): 1460–9.

[106] Halabe Bucay A. Clinical hypothesis: Application of AIDS vaccines together with thyroid hormones to increase their immunogenic effect. Vaccine 2007; 25(33): 6292-3.

[107] Hill AF, Polvino WJ, Wilson DB. The significance of glucose, insulin and potassium for immunology and oncology: a new model of immunity. J Immune Based Ther Vaccines 2005; 3: 5.

Received: September 22, 2009 Revised: October 2, 2009 Accepted: October 9, 2009