Stress related hormonal circuitry in Chagas disease

Ailin Lepletier[1], Silvina Villar[2], Ana Rosa Pérez[2],Alexandre Morrot[3], Wilson Savino[1]*

[1]Laboratory of Thymus Research, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil; [2]Institute of Immunology, Faculty of Medical Sciences, National University of Rosario and CONICET, Argentina; [3]Laboratory of Immunobiology, Paulo de Goes Institute of Microbiology, Federal University of Rio de Janeiro, Brazil

***Corresponding author:**

Wilson Savino

Laboratory on Thymus Research, Oswaldo Cruz Institute, Oswaldo Cruz Foundation.Ave. Brasil 4365, Manguinhos, Rio de Janeiro, Brazil.

Phone: 55 21 3865-8101

E-mail: savino@fiocruz.br- savino.w@gmail.com

1. Modulation of stress-related hormone production in *Trypanosomacruzi* infection

It is estimated that about 10-12 million of people are infected by *Trypanossomacruzi* in Latin America, within which 3 to 4 million only in Brazil. When left untreated, Chagas disease can be fatal, in most cases due to the cardiac sequelae, consequent of an exacerbated Th1 profile milleu. Despite the many efforts toward the control of vector transmission has largely reduced the numbers of acute cases, the mortality associated to Chagas disease is still alarming and many evidences point to an immunoendocrine imbalance in the basis of the development of its pathogenesis.

Disorders in the hypothalamic-pituitary-adrenal axis were frequently observed associated to *T. cruzi* infection, occuring in parallel to alterations in the systemic cytokine network. In particular, the significance of endogenous glucocorticoids (GC) levels for the course of thisinfection, relies on its imunossupressive activities, controlling the production of pro-inflammatory cytokines and its deleterious effects. In particular, TNF-α is the main mediator of the lethal course of experimental *T. cruzi* infection and an insufficient control of its production is expected to mediate harmful effects.

The immunosuppressive and anti-inflammatory actions of GCs are well recognized; they modulate genes involved in the priming of the innate immune response, while their actions on the adaptive immune response are to suppress cellular (Th1) immunity and promote humoral (Th2) immunity. GC also promote atrophy of the thymus gland and, triggering apoptotic signals in T cell precursors as well as in mature T cells. The relevance of anti-inflammatory effects of GC during infection is evident since deprivation of GC activity by adrenalectomy(ADX) significantly increased fever

and mortality during experimental sepsis, with GC administration reverting this situation.

Although being protective during *T. cruzi* infection, the GC imunossupression can be lethal by banish the host immune response against the pathogen. In this way, prolactin (PRL) has emerged as a stress-adaptation molecule necessary for controlling the negative side effects of GC and other immune or inflammatory mediators under stressful conditions. PRL is a polypeptide hormone synthesized and secreted from specialized cells of the anterior pituitary gland.An important role of prolactin has been well established in the modulation of the immune system activity and in the maintenance of the internal environment by regulation of osmotic balance in some species. Both circulating levels of PRL and GC are increased in many stressful conditions, a phenomenon related with the direct action of pro-inflammatory cytokines in the pituitary.

However, during the acute infection, this homeostatic mechanism is altered and the increased corticosterone is paralleled by decreased circulating PRL, demonstrating the immunosuppression induced by *T. cruzi* infection may be partially related to multiple endocrine changes involving the hypothalamus-pituitary axis and corresponding target endocrine glands. Further, in mammosomatotrophic cell line GH3; both GH and PRL secretion were decreased after *T.cruzi* inoculation, reflecting the diminished PRL concentrations in the pituitary glands of infected mice. This stress hormone imbalance was also evidenced observing the modulation of HPA axis. Besides the increased corticosterone serum levels, we detected a corticotropin-releasing hormone (CRH) impairment in the hypothalamus of acutely infected *T. cruzi* mice, with no significative changes in the amounts of circulating Adrenocorticotropic hormone (ACTH).

Although this GC/PRL imbalance takes to thymus atrophy and a higher parasitemia, it can be beneficial to the infected individuals, due the control of an exacerbated immune response and its lethal course, as previously referred. A systemic inflammatory milieu was evident in chagasic patients with severe myocarditis compared to healthy subjects, with enhanced levels of TNF-a and IFN-g. This was paralleled by a disrupted activation of the hypothalamus-pituitary-adrenal axis, characterized by decreased concentrations of dehydroepiandrosterone-sulfate (DHEA-s) and an unbalanced cortisol/DHEA-s ratio, reinforcing the view that severe Chagas disease is devoid of an adequate anti-inflammatory milieu, likely involved in pathology.

Because DHEA has been shown to protect against other protozoan infections, such as malaria and leishmaniasis a decrease in DHEA could weaken the ability to fight the *T. cruzi* infection. Furthermore, Santos et al.showed that DHEA supplementation exerted stimulatory effects on the cell-mediated specific immune response during *T. cruzi* infection, which decreased the parasite load in blood and tissues; this suggests that DHEA-s can be used as an adjuvant for the host's immune response against infection. Taken together, these results demonstrate high GC/DHEA-s ratio could exert a negative influence on the protective response, which has been observed in other chronic infections, such as tuberculosis human immunodeficiency virus (HIV). In schistosomiasis, the immunosuppressive effects of hydrocortisone and dexamethasone are counteracted by DHEA, which suggests that there is a tightly controlled balance in the secretion of these hormones that regulates the inflammatory response.

Explanations for the *T.cruzi* infection-associated immunoendocrine disturbances include several not mutually exclusive possibilities. Our recentdata demonstrate that changes in cytokine/ hormone levels can enhance or suppress the HPA axis, by acting at the hypothalamus-pituitary unit and/or at the adrenal glands. Infected TNF-R1 knockout

mice present increased synthesis of GCs through a mechanism related with the direct action of TNF-α as a potent modulator of steroidogenesis in adrenocortical cells. Further, corticosterone increase during infection,is related with an impaired secretion of PRL while hypothalamic-pituitary activity is dramatically enhanced after ADX, which causes changes in pituitary gland, and might play a role in PRL response during stress.Another possibility is that *T. cruzi* invasion in the endocrine microenvironment and the consequent inflammatory reactions and extracellular matrix enhanced deposition, may also lead to a transient HPA dysfunction, as observed with decreased PRL and GH detection in the pituitary of these mice. Corroborate with this hypothesisthe detection of parasites in the adrenal gland and their genomic product in both adrenal and pituitary glands of infected mice.

A better understanding on the relevance of immunoendocrine communication during infectious diseases, and how disturbances in the flux of information lead to neuroendocrine immune-related disorders, will provide important insights into mechanisms underlying this pathology.

2. Immunoneuroendocrine connectivity: The paradigm of the thymus-hypothalamus/pituitary axis

The interdependence of neuroendocrine and immune systems has been largely reported. These systems use similar soluble ligands to create and maintain physiological intra- and inter-system communication circuitries, which play a relevant role in homeostasis. Not only do the cells of the immune system produce classical hormones, but also the endocrine glands and nervous tissue synthesize release a variety of cytokines. The physiological action of such distinct molecular families is enabled by the expression of specific receptors detected in both the neuroendocrine and immune

systems. This cross-talk between neuroendocrine and the immune systems has been identified as a positive mechanism that regulates the immune response and favors the survival and health upon exposure to different environmental stressors.

The concept of bidirectionality between the neuroendocrine and immune systems is applied to analysis of thymic hormones, since these substances modulate the production of hormones and neuropeptides of the hypothalamus-pituitary axis and some of their target endocrine glands. Initial experiments revealed that neonatal thymectomy promotes developmental atrophy of female sexual organs and a decrease in the number of secretory granules in acidophilic cells of the adenopituitary. Additionally, it has been shown that production of sexual steroids is enhanced *in vivo* and *in vitro* by a single thymic hormone injection, thymulin that also stimulates corticotropin release in the rat anterior pituitary gland and the takes to the consequent activation of the HPA axis.

As the major part of the neuroendocrine system that controls reactions to stress and regulates many body processes including the immune system, the HPA axis activation create a circuitry essential to adaptive processes under adverse stimuli. HPA axis is regulated by circadian rhythm, stressful stimuli, and glucocorticoid negative feedback and its activation classically involves the release of CHR and vasopressin by hypothalamic cells and the increase of ACTH circulating levels. ACTH in turn acts on the adrenal cortex, which can either stimulates GCs or DHEA synthesis in response. Not only thymic hormones but many cytokines are now recognized as playing important roles in the HPA axis. In this respect, inteleucin-1 is a potent regulatory molecule, increasing the production of corticotropin-releasing factor and ACTH by hypothalamic neurons and pituitary cells.

The modulation of hypothalamus-pituitary hormones release by thymic hormones is also observed regarding the PRL and GH circuitry. Classically, the synthesis of both peptides is related with the activationof dopaminergic D2 receptors expressed in specialized cells localized in the adeno-pituitary and with pit-1 transcriptional activity. Alternatively, thymulin enhanced secretion of PRL and GC from rat anterior pituitary cells *in vitro*. This is in keeping with data showing that athymic nude mice exhibit significantly low levels of various pituitary hormones, including PRL, GH, LH, and FSH. However, contrasting results were reported using short-term cultures of pituitary fragments with no changes in GH levels but a significant inhibition of PRL release after thymulin treatment. Further, it has been demonstrated that IL-1 and TNF-a mediate LPS-induced GH release at the same time substance P, TNFα and IFNγ have emerged as novel modulators of PRL and PRLR expression in human skin, suggesting that the extrapituitary PRL synthesis is not under dopaminergic control.

As a part of bidirectional communication, the hormones released under stress conditions also influence the immune response. In this way, a great deal of data strongly indicate that the hypothalamic-pituitary axis plays a role in the control of thymus physiology. It has been largely reported that the imbalance of neuroendocrine circuitries alters the intrathymic development of T cells, eventually affecting the selection events and the migration of thymocytes to the T cell-dependent areas of peripheral lymphoid organs. Additionally, the interactions between thymocytes and microenvironmental cells are modulated by neuroendocrine hormones, which affect MHC expression, extracellular matrix-mediated TEC-thymocyte interactions and inter-TEC gap junctions. Through a mechanism apparently related with the increase of

adhesion molecules in thymocytes and ECM expression, GH and GCs control thymocyte traffic favoring the entrance of cell precursors into the thymus.

The thymic endocrine function and cytokine secretion by microenvironmental cells are also controlled by hormones and neuropeptides. The production of IL-1a and IL-1b by bovine nonepithelialthymicmicroenvironmental cells *in vitro* was increased by exogenous GH and by PRL. It was further demonstrated that secretion of IL-6 is also up-regulated by GH or by PRL treatment, an effect that could be abrogated by the use of the IL-1 receptor antagonist. Also, thyroid hormone and GCs modulate the thymulin secretion.

Pituitary and steroidal hormones oppositely influence the viability and proliferation of thymic cells. Both PRLR and GR are expressed in TECs and thymocytes, and the equilibrium of their signaling seems to be crucial for thymus homeostasis. While PRL *in vitro* treatment increasedthymocyte and TEC proliferation, GC inhibited it. Using lactogen-dependent rat Nb2 T-lymphoma cells, Witorsch and co-workers showed that GC-induced apoptosis, similarly to the effects of the steroids in thymocytes, is prevented by PRL administration. Subsequent studies designed to investigate the mechanism of apoptosis inhibition in this paradigm showed that PRL, in the presence or absence of GC, stimulated a rapid increase in expression of pim-1, bcl-2, bcl-xL, and XIAP (X-linked inhibitor of apoptosis), each a well-characterized suppressor of apoptosis. These data point to PRL as an important physiological anti-stress mediator in thymus.

It is noteworthy that a thymus-pituitary connectivity can also be seen under pathological situations. In this regard, an altered HPA axis has been reported in Chagas disease, which occurred in parallel with a severe thymic atrophy outcome.

3. The thymus is a target organ in Chagas disease

The thymus is a primary lymphoid organ in which bone marrow-derived T cell precursors undergo differentiation, according they migrate and interact with the thymic microenvironment, through a direct (cell-cell interaction) or indirect (mediated by soluble factors) mechanism. The thymocyte maturation involves sequential expression of various proteins and rearrangements of T cell receptor (TCR) genes. Once positively selected, mature $CD4^+$ or $CD8^+$ simple positive cells (SP) migrate to T cell-dependent areas of peripheral lymphoid organs, where they can be activated consequently proliferating and exerting their immune response.

Recent data show that normal thymocyte development and export can be altered as a result of an infectious disease. Specifically, in experimental models of Chagas disease, several alterations in lymphoid organs were observed, including the thymus where the parasite has been detected. The thymus atrophy is characteristic in the acute *T. cruzi* infection and results from the depletion of double positive (DP) thymocytes and the abnormal release of this immature subset into the immune periphery, resulting in more than 15 fold increase in DP cell numbers in subcutaneous lymph nodes. Once these cells express potentially autoreactive TCRs that are normally deleted in the thymus of uninfected mice, the presence of thisthymic subset in the periphery can be related with the development of chagasic myocarditis.

The alterations in lymphoid compartment are associated with changes of the thymic microenvironment in infected mice, comprising phenotypic and functional changes in TEC network, with enhancement in the deposition of cell migration-related molecules such the ECM proteins, fibronectin and laminin, as well as the chemokines CXCL12 and CCL21 and its respective receptors, which correlates with a higher

fibronectin-driven migration of DP thymocytes, and the abnormal raise of immature DP cells in lymph nodes. Recently, we demonstrated that trans-sialidase was able to modulate the adhesion of thymocytes to thymic epithelial cells and their migration toward extracellular matrix. When associated with the detection of increased percentages of blood DP subsets paralleled by the augment of antibody titters against TS in chagasic patients with chronic cardiomyopathy, these findings raise the possibility that TS could influence the escape of immature thymocytes in Chagas disease.

In the thymus, potentially autoreactive lymphocytes bearing high affinity TCR that recognize autoantigens presented by TECs, are induced to undergo apoptotic cell death during the negative selection process. Intrathymic expression of tissue-restricted antigens (TRAs), activated by the autoimmune regulator (Aire) protein in TECs, has an imperative role in this event. Previous studies have shown that the disruption of normal thymic architecture affects the expression pattern of autoantigens by TEC and functionality of thymus. Recently, we have determined whether the changes of the thymic microenvironment seen following *T. cruzi*infection, would also lead to an altered intrathymic negative selection of the T-cell repertoire. We showed that the expression of Aire and TRAs was readily detected in similar levels in infected mice and their respective uninfected controls. Although the intrathymic checkpoints necessary for thymic negative selection are present in the acute phase of Chagas disease, we found that the DP cells released into the periphery acquire an activated phenotype similar to what is described for activated effector or memory single-positive T cells.

The thymic atrophy triggered by *T. cruzi*seems to be complex, with many host-derived molecules likely being involved. For example, thymic atrophy is not seen in *T. cruzi*-infected galectin-3 knockout mice while ATP induces an increase in plasma membrane permeabilization and cellular death in DP thymocytes collected from

infected mice during the atrophy phase. On the other hand, the parasite-derived trans-sialidase is involved in the generation of intrathymic T-cell death. Yet, recently we demonstrated that thymics alterations during infection are associated with a stress-hormone imbalance characterized by the augmented GCs and decreased PRL levels. Immature thymocytes are very sensitive to corticosteroids, since elevated levels during stress conditions cause a profound cortical involution, with the induction of apoptosis in DP subset, mediated by caspase activation. Since PRL seems to counteract many imunossupressive effects mediated by GC, stimulating a rapid increase in expression of pim-1, bcl-2, bcl-xL, and XIAP (X-linked inhibitor of apoptosis), each a well-characterized suppressor of apoptosis, this impairment of PRL levels seems to acts as a permissive factor to the deleterious effects of GC upon the thymus. Indeed, the reestablishment of circulating PRL in later acute infection restored thymus cellularity, diminishing the loss of DP by apoptosis and inhibiting the activation of caspase-3.

Importantly, reestablishing systemic PRL not only prevented thymic atrophy, but also significantly decreased the numbers of DP cells in the periphery of the immune system, demonstrating that PRL-mediated thymus protection also influences in the abnormal export of these immature T cells.

4. Systemic versus intrathymic stress-related hormones in Chagas disease

Thymus-pituitary similarities for cytokines and hormone productions have been demonstrated. Intrathymically produced hormones seem not only act as endocrine messengers from the pituitary gland, but also appear to represent autocrine and paracrine factors involved in the general regulation of the thymus.

In addition to the endocrine function of GC and PRL upon thymocytes and thymicmicroenvironmental cells, both hormones are produced intrathymically, and likely act locally through autocrine and/or paracrine loops.

The thymus is the lymphoid organ that shows the largest response to hormonal fluctuations. Changes levels of stress hormones have a profound effect on the thymus, resulting in a marked reduction in cellularity of the cortex. Mice treated with *T. cruzi* demonstrated a disrupted thymic homeostasis by altering intrathymic and systemic stress-relate hormone, wherein PRLand GCs exert a major consequences upon the normal process of intrathymic T cell development

Both GC and PRL are intrathymically produced, during infection, the intrathymic PRL production was augmented as being the medulla thymic epithelial cells are the main source, on the other hand, an infected thymus decrease in the local production of corticosterone, demonstrating that the intrathymic hormonal circuit is altered during infection. The thymic atrophy is associated to an imbalance of their respective receptors PRLR and GR. In *in vitro*, studies freshly DP thymocytes isolated from infected mice showed a decrease in GR and increased in PRLR gene expression. These cells also exhibited a lower apoptosis after dexamethasone challenge, while likely were not affected when were treated with prolactin. Considering that GC induce apoptosis and inhibit DP thymocyte proliferation and, contrary, PRL administration prevents these effects, it seems plausible that a local imbalance of GR–PRLR crosstalk underlies thymic involution occurring in acute *T. cruzi* infection, suggesting these receptors may interact influencing DP viability.

T. cruzi-related thymic changes are associated with systemic and intrathymic PRL-GC hormonal imbalances. In addition, some evidences suggest that PRL and GC

are potent mediators of thymic involution. In normal mice, BRC treatment (potent D2 agonist that inhibits the release of pituitary PRL) increased the intrathymic contents of PRL without changes in both systemic and intrathymiccorticosterone levels, demonstrating that each circuitry (systemic and thymic) acts independent on each other. Nevertheless, infected animals treated with BRC increased systemic GC levels and decreased thymocyte number. These finding suggest that the systemic GC production likely depend in part on pituitary PRL secretion during infection. However, we did not detect any alteration of PRL levels in infected mice lacking circulating GCs. It seems that although in infected mice PRL diminution acts as a permissive factor to the increase in GC synthesis, the PRL impairment are not related, at least directly, to the increase of GC systemic levels.

The administration of metaclopramide (MET, used to increased PRL systemic levels) in acute infected mice, reestablished thymic atrophy without altering systemic GC levels, demonstrating a direct effect of PRL upon thymocytes. Interesting, PRL intrathymic levels were decreased after MET treatment, suggesting that thymus recovered after MET is mostly related with circulating PRL than with autocrine/paracrine effects. The relevance of systemic PRL upon locally produced one relies on the fact that besides several approaches have demonstrated the PRL expression by the thymic microenvironment, whether this peptide is actually secreted is not clear. Besides, medullary thymic epithelial cells are the main intrathymic source of PRL during acute infection. Even if they secreted PRL, its anti-apoptotic effects wouldn't be available to DP localized in the cortical area.

This impairment of intrathymic PRL paralleled by increased systemiclevels in infected mice, suggest that the availability of intrathymic hormones is controlled by a negative feedback loop based on its plasmatic concentrations. Corroborating with these

data, a transient increase in the intrathymic contents of thymulin observed after adrenalectomy and/or gonadectomy, seems to correspond to a TEC response to the fall in circulating levels of biologically active thymulin, secondary to the appearance of its natural inhibitor. Such a feedback circuit with increase in thymulin production had been previously shown in mice treated with antithymulin monoclonal antibodies.

The recent development of new tools to investigate thymic atrophy in infection processes suggests that a new research focus on this old topic may be taken into account and exploring the autocrine/paracrine circuitries seems to be an important clue.

5. Immune and stress-related hormonal disturbances in Chagas´ disease: possible consequences upon pathology.

HPA axis stimulation mediated by diverse cytokines like IL-1β, TNF-α and IL-6 is evidently important for the immuno-regulation and survival during diverse infections. This endocrine response aims to inhibit cell-mediated immunity suppressing IL-2 and IFN-γ production and promoting Th2-cytokine production. Regarding *T. cruzi* infection, comparative studies carried out between susceptible and resistant strains of mice showed that the susceptibility to the disease depends on the appropriate timing and magnitude of the HPA axis activation. An early and balanced endocrine response are linked to better course of infection, while a delay and later deregulated GC response are clearly linked to susceptibility.

As previously mentioned, beyond their protective effects on peripheral cell-mediated immunity, the enhancement of corticosterone levels also results in thymus atrophy and a marked depletion of DP cells by apoptosis. In this context, TNF-α emerge as an important and complex player in the immune-endocrine deregulation observed during infection. It is involved in the development of cardiac tissue damage, but also

contributes to prolong survival. TNF-α is, together with IL-1β, involved in the activation of HPA axis, and the evidence indicates that a subtle balance between corticosterone response and TNF-α and IL-1β production is necessary for an efficient defense against *T.cruzi* infection, since infected and adrenalectomiced animals have a better control of parasite spread, but develop a severe TNF-α mediated inflammation and reduced their survival time. In addition, we recently showed that TNF-α may influence corticosterone production, not only through the HPA axis, but also acting as potent modulator of steroidogenesis in adrenocortical cells, reinforcing diverse *in vitro* evidences that indicated that adrenal secretion of glucocorticoids can be modulated *in situ*, in an ACTH-independently fashion, by cytokines such as TNF-α, IL-6 or IL-1β. TNF-α signalling may lead to the activation of various MAPK networks, contributing to the induction of NF-kB and AP-1 nuclear factors and implicated in the expression of steroidogenic enzymes. During *T. cruzi* infection, TNF-α intra-adrenal expression and NF-κB and AP-1 activation was increased, and notably blunted in infected-TNF-R1 KO mice. Strikingly, the accumulation of mRNAs for steroidogenic acute regulatory(StAR)protein and cytochrome P450 enzymes and the ratio HSD1-11β/HSD2-11β were significantly more augmented in TNF-R1 KO infected animals, and these finding correlates with the more increased corticosterone levels that exhibited KO mice compared to wild type after infection (Villar, Ronco et al. 2013)(Perez, Roggero et al. 2007).These *in vivo* findings point out that TNF-α throughout TNF-R1 can affect negatively GC release at adrenal level. Moreover, adrenal contents of IL-6 and IL-1β mRNAs are also elevated during *T. cruzi* infection, and likely exert a stimulatory effect on steroidogenesis, however this still needs to be proven. Accordingly, these finding revealed the establishment of an intra-adrenal

immunoregulatory circuit potentially involved in the pathogenesis of murine Chagas disease.

In human Chagas disease, the immune-endocrine imbalance is also apparent. Chagasic patients with severe myocarditis showed a marked systemic inflammatory milieu, with enhanced levels of TNF-α, IL-6 and IL-1β compared to healthy subjects. This scenario was paralleled by a disrupted activation of the HPA axis, characterized by decreased concentrations of DHEA-s and an unbalanced cortisol/DHEA-s ratio, reinforcing the view that severe human Chagas disease is devoid of an adequate anti-inflammatory environment that favours pathology. Recent evidence obtained in mice and humans demonstrated that during acute or chronic *T. cruzi*infection, DP cells could be also tracked in secondary lymph organs showing an activated phenotypeand raised new questions about the relevance of this population in the pathogenesis of this parasitic disease and their possible links with immune-endocrine alterations.

In keeping with this notion, we observed that extrathymic DP T cells positively correlated with the circulating levels of TNF-α and with the cortisol/DHEA-s ratio in the overall study population, and within those chagasic patients having cardiopathy. By contrast, a negative correlation between extrathymic DP T cells and DHEA-s was found in the overall population and also in cardiac patients. At the same time, PRL reestablishment in infected mice decreased peripheral DP amounts without altering corticosterone levels. This raises the question whether there is a cause/effect relationship between immunoendocrine abnormalities and the levels of circulating extrathymic DP T cells linked to clinical progression in humans. In this vein, it is noteworthy that DP cells seen in peripheral lymphoid organs express high densities of ECM and chemokine receptors. Among these abnormally released DP cells in the periphery, we found lymphocytes expressing potentially autoreactive TCRs, which are

normally deleted in the thymus of uninfected mice. We have previously showed once these immature T lymphocytes escape from the thymic central tolerance process and migrate to the lymph nodes, they eventually differentiate into mature $CD4^+$ or $CD8^+$ cells.

Chronic stimulation of pro-inflammatory cytokines may influence the activate state of DP T cells, and at the same time, the cortisol/DHEA-s ratio imbalance acts as permissive scenario to myocarditis development. Further investigations concerning how endocrine imbalances may influences the immune response and particularly the phenotype and effector functions of extrathymic DP cells may help to elucidate the contribution of these cells to the etiology of chagasiccarditis.