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Brain-Thymus Connections in Chagas Disease

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Abstract
Background: The brain and the immune systems represent the two primary adaptive systems within the body. Both are involved in a dynamic process of communication, vital for the preservation of mammalian homeostasis. This interplay involves two major pathways: the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system. ***Summary:*** The establishment of infection can affect immunoneuroendocrine interactions, with functional consequences for immune organs, particularly the thymus. Interestingly, the physiology of this primary organ is not only under the control of the central nervous system (CNS) but also exhibits autocrine/paracrine regulatory circuitries mediated by hormones and neuropeptides that can be altered in situations of infectious stress or chronic inflammation. In particular, Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi (T. cruzi)*, impacts upon immunoneuroendocrine circuits disrupting thymus physiology. Here, we discuss the most relevant findings reported in relation to brain-thymic connections during *T. cruzi* infection, as well as their possible implications for the immunopathology of human Chagas disease. ***Key Messages:*** During *T. cruzi* infection, the CNS influences thymus physiology through an intricate network involving hormones, neuropeptides, and pro-inflammatory cytokines. Despite some uncertainties in the mechanisms and the fact that the link between these abnormalities and chronic Chagasic cardiomyopathy is still unknown, it is evident that the precise control exerted by the brain over the thymus is markedly disrupted throughout the course of *T. cruzi* infection.

Chagas Disease and the Thymus and Brain Involvement

Chagas disease is a neglected infectious disease of great importance in Latin America, caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). The infection can be transmitted by triatomine vectors, food, or beverages contaminated with live parasites, organ transplantation, blood transfusions, and also vertically. These three latter forms of transmission are responsible for Chagas disease spreading in non-endemic areas,

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including the USA, and many countries of Europe, Asia, and Oceania [1]. Approximately 7 million people worldwide are affected with this disease. The infection courses with a short period in which parasites are easily found in blood and in almost all tissues. Following the acute phase, infected individuals enter into a long and asymptomatic phase which can remain silent for the rest of life. However, after many years, nearly 30% of infected patients develop the chronic clinical forms of Chagas disease, such as the chronic Chagasic cardiomyopathy (CCC) and megavisceras, including esophagus and colon. In addition, there is increasing evidence on the occurrence of brain affectation during both acute and chronic *T. cruzi* infection [2]. In fact, the central nervous system (CNS) is the most frequent site of *T. cruzi* reactivation in HIV-infected patients with immunosuppression [3]. Meningoencephalitis has been reported during the acute infection, more frequently in children under 2 years of age and it is almost always fatal [4]. It has been shown the presence of parasites in diverse brain areas, mainly the meninges, cerebral and cerebellar cortices and in other parts of the CNS as the hypothalamus and pituitary gland, as well as inflammatory infiltrates and in situ production of pro-inflammatory cytokines, promoting neuroinflammation [2, 5]. In addition, it was demonstrated that *T. cruzi* can infect endothelial cells, microglia, astrocytes, and neurons [6]. During the chronic phase of Chagas disease, behavioral and cognitive impairment, as well as sensorial and motor deficits are often the symptoms of CNS involvement. In general, CNS parasitism occurs in the acute phase or after parasite reactivation in immunosuppressed individuals, in parallel with the peak of parasitemia [5, 7–10].

The pathogenesis of Chagas disease is still under investigation and debate. One current hypothesis supports that damage to the cardiac or enteric tissues (and potentially the brain) is mediated and sustained by chronic inflammatory reactions triggered by parasite persistence, whereas the autoimmune hypothesis of the disease is supported by data showing the occurrence of autoimmune events, caused either for bystander activation or by molecular mimicry. However, both hypotheses are not mutually exclusive [11]. Moreover, the impairment of the autonomic nervous system and other neurohormonal circuits observed in patients with Chagas disease [12, 13] may contribute to ongoing tissue damage that leads to cardiac, digestive, and also nervous system dysfunctions.

Whatever the case, it is well established that neuroendocrine mechanisms are relevant for the susceptibility and course of the disease [14, 15]. Particularly, in animal models, the infection greatly affects the thymus, causing structural and functional alterations of the gland, mainly characterized by an intense atrophy caused by massive cell death, an abnormal thymic output of immature, activated and IFN-γ producing cells with autoreactive potential, alterations in the double-negative (DN) population and depletion of regulatory T-cells [16]. Also, bone marrow aplasia and a decrease in common lymphoid progenitors appear before thymic alterations [17].

Several studies indicate that *T. cruzi*-driven thymic atrophy is caused by an immunoneuroendocrine imbalance [5, 14], added to the fact that thymic abnormalities can persist during the chronic phase of infection [18–20]. These findings increase the suspicion that thymic affectation might provoke defects in the mechanisms responsible for central tolerance and that could be related to the establishment of CCC, although this issue remains uncertain [19, 21]. Anyhow, the study of thymus-brain interrelationships that take place in the context of Chagas disease could provide interesting clues to disclose important issues related to pathophysiological and disturbing effects upon the thymus, which may be of relevance in terms of both thymic selection and T-cell mediated immune response. Accordingly, we discuss herein the most relevant findings reported in relation to brain-thymus connections during *T. cruzi* infection, as well as their possible implications for the immunopathology of human Chagas disease.

The Thymus: Immunoneuroendocrine Aspects

During the last years, several advances in the field of immunoneuroendocrinology have established that the CNS and the immune system are intimately related in both health conditions and disease [22]. In particular, the thymus, where T lymphocytes mature, displays functional networks with different CNS structures. Moreover, the brain seems to share with the thymus common functional principles in terms of recognition, memory, and learning, as proposed by Sanchez-Ramón and Faure [23]. These networks seem to be particularly important during perinatal life, when the thymus and certain structures of the neuroendocrine system seem to reciprocally influence the maturation of each other [24]. During the adult life, neuroendocrine axes undoubtfully control thymus physiology [25].

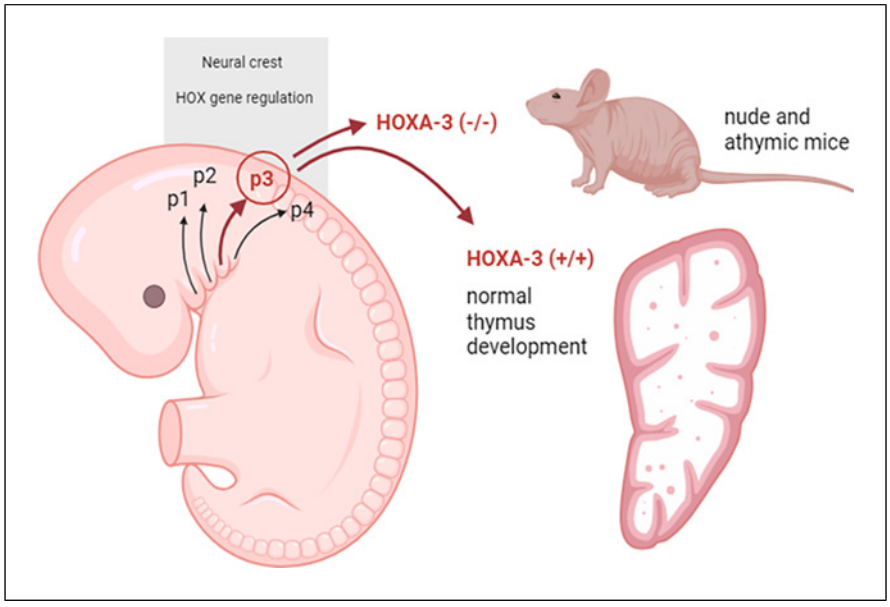

Fig. 1. Embryology of thymus-brain connections. The nervous system develops from the neural ectoderm. The outermost cells of the neural plate are the neural crest cells, and as the neural tube sinks and fuses, the neural crest cells separate off and migrate away. Some of them migrate into the pharyngeal arches and proliferate. The cranial neural crest plays a critical role in the development of the pharyngeal arches and pouches (called p1, p2, p3, and p4, respectively), initially by providing the mesenchymal cells which populate this region. The pharyngeal arches and pouches are transient embryonic structures that give rise to many of the tissues and organs in the head and neck region of vertebrates. The thymus originates from the third pharyngeal pouch (p3) and the epithelial primordium of the thymus expands from pharyngeal endoderm. Experimental studies have established the importance of mesenchymal derivatives from the neural crest cells in thymus development. For example, and as shown in the figure, interfering with crest cell proliferation and function, as result of the deletion of the HOXA-3 gene, inhibits thymic development and in consequence, HOXA-3 mutant homozygotes mice are athymic.

Brain-thymus communication depends on some functional similarities, explained in part by their common embryonic origin (Fig. 1) [26–29], and a complex network of shared factors [22, 26]. Both thymic epithelial cells (TECs) and thymocytes, as well as other thymic microenvironmental cell components like fibroblasts or macrophages, can produce a large number of cytokines and chemokines. Additionally, some of them can produce and release certain hormones, neurotransmitters and neuropeptides typically produced by "classical" neuroendocrine structures. The effects of these thymic cytokines, hormones or neural products are locally limited, providing support for the T-cell maturation processes acting through specific receptors, and their local functions might differ considerably from those in the periphery [25, 30]. Lastly, despite the various studies highlighting the significance of the neuroendocrine regulation of the thymus, there is so far no evidence that cytokine, hormones (perhaps with the exception of thymulin [24]), neurotransmitters or neuropeptides secreted within the thymus can directly affect brain functions.

Sympathetic Control of the Thymus and Its Potential Implications in Chagas Disease

The main neural regulation of thymic function is provided by a sympathetic neural input through its main neurotransmitter, norepinephrine (NE). Diverse studies carried out in animals have evaluated the thymus innervation observing the presence of nerve fibers in each thymic compartment, including the capsule, the subcapsular region, the cortex, the cortico-medullary junction, and the medulla. Therefore, nerve fibers are considered intrinsic constituents of the thymic microenvironment and thought to regulate thymocyte development by the exposure to neurotransmitters and neuropeptides [31, 32]. Nerve fibers of the sympathetic nervous system (SNS) enter the thymus along with blood vessels. Noradrenergic nerve activity in the thymus can be controlled by signals from the CNS due to the connectivity of the sympathetic ganglia from which they originate [32]. The noradrenergic fibers are predominantly found in the cortex, co-localizing with immature $CD4^+CD8^+$

double-positive (DP) thymocytes, also showing a slightly higher density near the cortico-medullary junction. This type of fibers is also found adjacent to TECs in the deep cortex and in the medulla [32, 33], where the more mature simple-positive (SP) thymocytes are confined. Moreover, microscopic studies showed close contacts between noradrenergic nerve fibers and thymocytes, while mainly β2-adrenergic receptors have been detected in both TECs and thymocytes at various stages of maturation and differentiation, supporting the sympathetic control of the T-cell development [34–36]. The NE released at nerve endings seems to exert a variety of effects through β2-adrenergic receptors ranging from suppression of thymocyte chemotaxis, diminution of mitogen-induced thymocyte proliferation, increase of IL-6 production by TECs, modulation of the selection process, and the induction of apoptosis in thymocytes [31]. The α1-AR seems to promote the differentiation of DP to CD8+ SP but prevents the differentiation of positively selected thymocytes toward the CD4+ SP phenotype [37].

There is no neuroanatomical evidence to support a parasympathetic innervation of the thymus [38]. Even though acetylcholinesterase (AChE)-positive nerve profiles, identified through AChE or choline acetyltransferase histochemistry, have been observed in the capsule, interlobular septae, subcapsular regions near TECs, and cortico-medullar spaces close to thymocytes [39], subsequent studies involving sectioning of the cervical vagus did not alter the AChE staining in the thymus. This indicates that AChE does not originate from cholinergic fiber terminals [38, 40] but is produced and released by lymphocytes and microenvironmental cells [37]. Additionally, it has been shown that diverse neurotransmitters can be synthesized and released by lymphoid and microenvironmental cells [37]. The graphical representation of these findings is succinctly presented in Figure 2.

Several studies have demonstrated that the hypothalamus-pituitary-adrenal (HPA) axis is activated during the acute phase of *T. cruzi* infection (as explained later), and since this axis operates in concert with the SNS, it is highly likely that sympathetic circuitries are activated in the early stages of the infection. Indeed, changes in the SNS have been noted in the spleen during the peak of parasitemia in mice [15]. Notably, there is a noteworthy decrease in splenic NE levels, which corresponds to a reduction in noradrenergic nerve fibers, although plasma levels remain unchanged. Moreover, acute infection appears to result in a significant reduction in NE contents within the thymus, mirroring the atrophy observed in the organ (Roggero et al. [31] personal communication). Yet, a putative role concerning the loss of thymic sympathetic innervation in the atrophy or in thymocyte alterations that occur during acute or chronic infection by *T. cruzi* remains to be determined. Despite the underlying molecular mechanisms that lead to the reduction of NE in the spleen of *T. cruzi*-infected mice are still unclear, indirect evidence suggests that pro-inflammatory cytokines such as IL-6 might down-regulate the expression of tyrosine hydroxylase in sympathetic endings [15], suggesting that a similar mechanism may be operative at thymic level since this cytokine is known to be upregulated during acute and chronic *T. cruzi* infection [41, 42].

Immunoreactivity against several neuropeptides, such as the neuropeptide Y, the vasoactive intestinal polypeptide, the substance P, the calcitonin gene-related peptide, has been described in human and animal thymuses [43–46], but nowadays there is not enough evidence that these neuropeptides are associated with any sensory feedback from the thymus gland, probably being released by TECs and thymocytes. Different studies have shown that neuropeptides can influence thymocyte maturation, mainly affecting cytokine production [47], migration [48], regulatory T-cell development [49], and corticosterone-induced death [50–52]. However, in a similar way to what happens with catecholamines, there are no studies available highlighting its role during thymic atrophy caused by *T. cruzi*. Future studies on this issue could allow the development of strategies to protect the thymus during infection.

The Influence of HPA Axis upon Thymic Functionality during *T. cruzi* Infection

It is well established that thymus physiology is controlled by several hormonal axes, as the HPA axis [53], being the unit "hypothalamus-pituitary gland" located just above the brainstem. Upon the activation of the HPA axis by stressful inputs (including infectious stress), adrenal glands release glucocorticoids (GC; cortisol in humans and corticosterone in rodents), which exert well-known pro-apoptotic effects in thymocytes [54, 55].

In the context of infections, pro-inflammatory cytokines like IL-1β, IL-6, and TNF-α released by activated immune cells gain access to the CNS and act at the paraventricular nucleus of the hypothalamus to release corticotropin-releasing hormone. This neurohormone reaches the anterior pituitary, via the hypophyseal-portal circulation, to stimulate the release of adrenocorticotropic hormone (ACTH) into the circulatory system

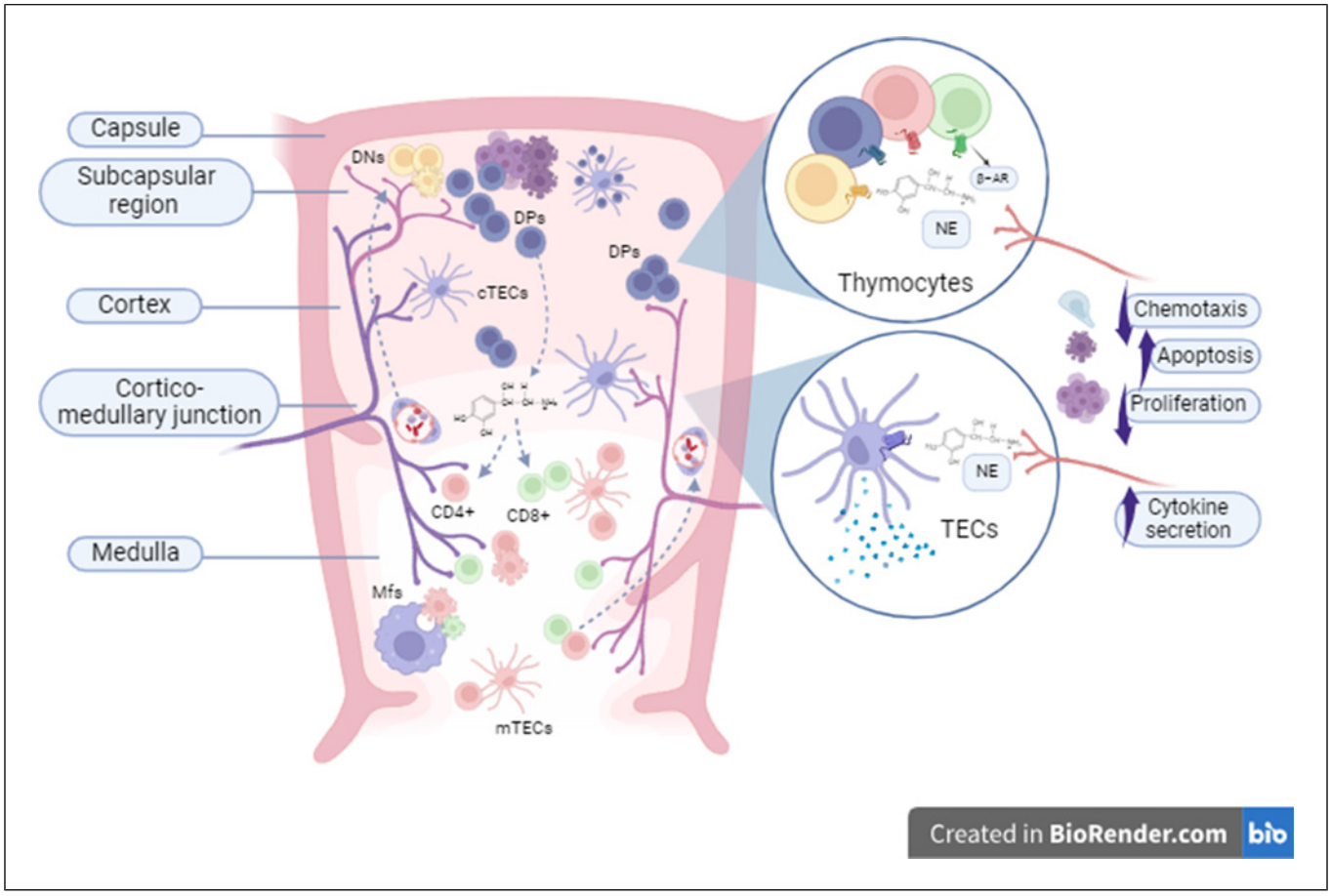

Fig. 2. Thymic microenvironment and cell populations that can be modulated by sympathetic innervation and NE in both physiological and Chagas disease contexts. The figure also shows schematically thymic sympathetic innervation and the general process of thymocyte differentiation. Sympathetic fibers enter to the thymus following cortico-medullary vessels and expand through the capsule, the subcapsular region, the cortex, the cortico-medullary junction and the medulla, secreting NE. Bone marrow-derived thymocyte precursors enter the organ through capillaries at the cortico-medullary junction and migrate toward the outer cortex where they proliferate, where differentiate to CD4-/CD8-double-negative cells (DN), which evolve to express TCR as well as CD4+ and CD8+ becoming double-positive cells (DP). DP thymocytes are positive selected, migrating toward the medulla, where some of them will die by negative selection. Mature CD4+ or CD8+ simple-positive thymocytes will eventually exit the thymus. Both thymocytes and TECs express mainly β-adrenergic receptors (β-AR), thus all differentiation process and positive and negative selection are influencing by sympathetic signals. mTECs, medullary thymic epithelial cells; CTECs, cortical thymic epithelial cells; Mfs, macrophages; NE, norepinephrine.

resulting in the adrenal secretion of GC to counteract the negative effects of acute inflammation. In the thymus, the immature DP thymocytes are major targets of GC-enhanced levels, as this population undergoes high levels of apoptosis and decreased proliferation. The glucocorticoid receptor (GR), a ligand-activated transcription factor, is expressed in the cytoplasm of thymocytes, being able to bind to the steroid hormone, and translocate to the cell nucleus, where it modulates the expression of target genes. The density of GRs is increased in DP thymocytes compared with mature single-positive thymocytes, which explains the greatest resistance seen for the latter.

In the context of animal models of acute Chagas disease, a systemic enhancement of IL-1β, IL-6, and TNF-α elicits the activation of the HPA axis [5, 14, 41]. This cytokine-driven enhancement of GC triggers the apoptotic program in DP thymocytes acting via the GR that leads to the activation of caspases 8 and 9 [56]. Likewise, blockade of GR transduction signals with RU486 prevented not only DP thymocyte apoptosis [41] but also the activation of caspases 8 and 9 [56].

Another aspect deserving comment is the fact that GC signaling participates in setting the TCR avidity thresholds that determine whether developing thymocytes survive or die, and therefore help mold the antigen-specific and auto-tolerant T-cell repertoire [55, 57]. By now, we do not know whether changes in GC levels during *T. cruzi* infection alter per se the repertoire. However, diverse changes in the peripheral T-cell repertoire were reported after *T. cruzi* infection [18, 20, 21, 58, 59]. The most striking is that potentially auto-reactive DP T-cells can be tracked in the heart and lymphoid organs of infected animals and also in the blood of individuals with chronic Chagas disease [16], suggesting that they have bypassed the events of negative selection. Yet, another study carried out in mice suggests that negative selection itself is still operative in the thymus of infected animals [19], thus suggesting that the leak of immature thymocytes occurs before negative selection events.

The intrathymic expression of GR is also altered during *T. cruzi* infection, adding a new level of complexity [60]. These findings open an interesting area of study in the context of *T. cruzi* infection, necessary to understand to what extension the alterations observed in thymocyte selection are a direct (or indirect) consequence of changes in the levels of GC or their receptor.

As mentioned earlier, both the hypothalamus and pituitary gland can be functionally affected during *T. cruzi* infection, as these organs can be parasitized by amastigotes, infiltrated by immune cells, and experience alterations in their levels of extracellular matrix deposition [5]. However, whether these alterations affect corticotropin-releasing hormone and ACTH release remains unknown, but interestingly, GC synthesis may be ACTH-dissociated in the late phase of this parasitic infection, with evidence suggesting that actions mediated by IL-6 at the HP unit, as well as by IL-1β at adrenal level, are involved in HPA axis uncoupling [61, 62].

Other Pituitary Hormones Regulate Thymic Functions during *T. cruzi* Infection

Growth hormone (GH) and prolactin (PRL), both considered stress-related hormones, act as modulators at thymic level, having opposing actions of GC on the viability and proliferation of thymocytes and their receptors, both expressed by TECs and thymocytes [63–65]. Despite thymocytes express GH and PRL receptors independently of the maturation stage, their signalings are especially important for the survival and proliferation of early DN T-cell precursors and, for the protection of DP-cells from GC-induced apoptosis [66]. Furthermore, both hormones enhance the proliferation of cultured TECs, the secretion of thymulin, and stimulate the expression of extracellular matrix ligands and receptors, with the consequent increase in the adhesion of thymocytes [65, 67–70]. Interestingly, during acute *T. cruzi* infection in mice, besides the increase in systemic GC levels, a decrease in plasma PRL contents is observed, further stimulating the loss of immature thymocytes. The diminished PRL circulating levels are probably due to the parasite invasion of the pituitary gland, local inflammatory reactions, and the enhanced extracellular matrix deposition [5]. This notion is also supported by the fact that GH/PRL-secreting GH3 cells reduce the secretion of both GH and PRL after *T. cruzi* infection. Moreover, both GC and PRL are produced intrathymically and showed differential modulation during the infection, interfering with each other through autocrine and/or paracrine loops [60]. In this way, despite the systemic GC/PRL imbalance may contribute to intensifying the thymic atrophy observed during experimental infection, signals mediated by PRL receptors on DP thymocytes tend to counteract GC-related deleterious effects on cell survival [60]. Furthermore, the enhancement of GH secretion by metoclopramide treatment, preserved the gland from atrophy and notoriously, significantly avoided the release of immature and potentially autoreactive DP cells to the periphery [60].

In addition to GH, other hormones of the somatotropic axis as IGF-1, ghrelin, and somatostatin are expressed in the thymus, where they are involved in the general process of intrathymic T-cell development [71]. IGF-1 mediates a large number of the effects of GH upon the thymus: the enhancing effects of GH upon thymulin production, extracellular matrix expression, and adhesion of developing thymocytes to TECs can be abrogated by treating cells with anti-IGF-1 and IGF-1R antibodies [69]. In vitro findings point out that *T. cruzi* infection in the mammosomatotropic cell line GH3 induces a diminution in the hormone synthesis, suggesting that in vivo, this deficiency may favor a more permissive environment for the induction of DP cell apoptosis. It is still necessary to evaluate if strategies to supplement GH levels could avoid the atrophy of the gland during *T. cruzi* infection, since in individuals with GH deficiency, recombinant GH treatment restores thymic function, at least in terms of T-cell output [70]. In addition, ex vivo studies suggested that GH protects against the parasite [72]. The L-cell murine fibroblast line, when infected and preexposed to serum

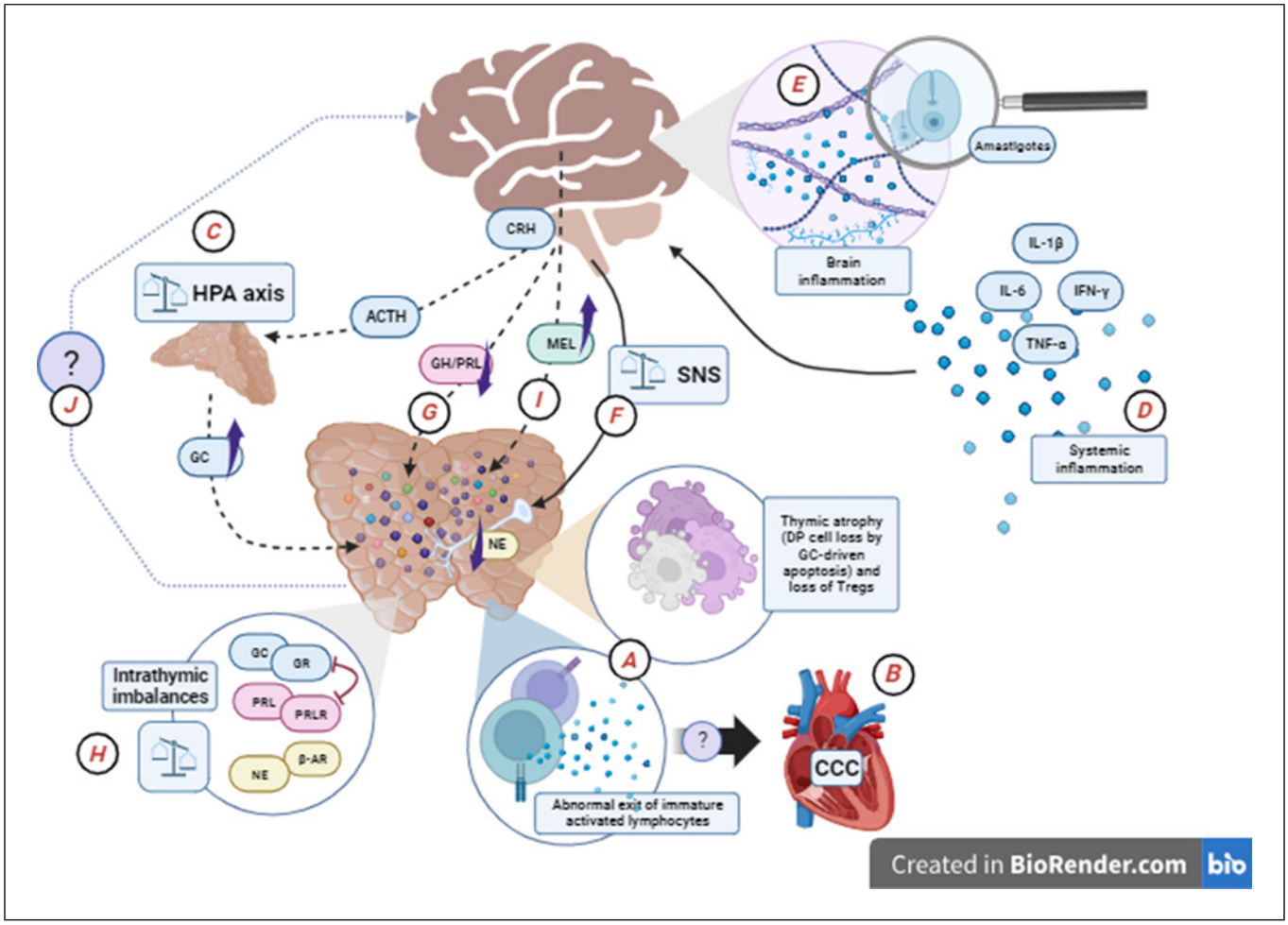

Fig. 3. Brain-Thymus connections in the context of Chagas disease. In rodent models of Chagas Disease, the control exerted by the brain over the thymus is markedly disrupted influencing the atrophy of the gland. Thymic atrophy is primarily characterized by extensive cell death among DP thymocytes, an unusual thymic output of immature, activated, and IFN-γ-producing cells with the potential for auto reactivity, and a reduction in the thymic Tregs (**A**). These findings raise the critical questions of whether the observations made in the thymus of *T. cruzi*-infected rodents can be extended to humans and whether they deserved significance in the development of Chagas cardiomyopathy (**B**). Likewise, in these animal models, the CNS seems to influence the thymus physiology mainly through complex systemic and local networks achieved by hormones, neurotransmitters, and pro-inflammatory cytokines. The activation of HPA axis (**C**) is a consequence of systemic inflammation (**D**), and in some cases probably fueled by brain neuroinflammation (**E**), being the GC the primary causative agents of DP thymocyte apoptosis. In parallel, the SNS could be activated by the infectious-driven stress process, but a decrease in the intrathymic contents of NE was reported (**F**). PRL and GH hormones have anti-apoptotic effects upon thymocytes axis, being the GH/PRL axis downregulated during infection (**G**). Furthermore, there are intrathymic circuits influenced by both systemic and/or locally produced immuneneuroendocrine ligands and their respective receptors that tend to counteract the deleterious effects of GC (**H**). The pineal-thymus axis seems to be also activated during the infection, with possible anti-apoptotic effects of MEL (**I**). Lastly, the extent to which thymic products could impact brain functions, whether via soluble factors or axonal retrograde transport, still remains a notable gap in our current understanding (**J**). CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone; GC, glucocorticoids; GR, glucocorticoid receptor; PRL, prolactin; PRLR, prolactin receptor; GH, growth hormone; NE, norepinephrine; β-AR, beta adrenergic receptor; Tregs, thymic regulatory T cells; MEL, melatonin; IL-6, interleukin-6; IL1β, interleukin-1 beta; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha.

with significantly elevated levels of GH, regardless of higher or lower IGF-1 levels, exhibited superior control over parasite growth compared to cells preexposed to regular serum [72]. Importantly, this effect does not seem to be mediated by cytokines such as TNF-α, IL-6, or IL-1β [72].

So far, data regarding somatotropic axis function during human acute phase of Chagas disease or upon their thymic implications are still lacking. Yet, in patients with chronic Chagas disease bearing chronic cardiopathy, alterations in the GH/IGF-1 axis were reported [13], together with a decrease in the sommatotropic axis activation in response to glucose or insulin in individuals, compare to healthy subjects [73]. These findings collectively unveil the presence of an intricate intrathymic network of stress-related hormones, actively participating in the modulation of signals that regulate the survival of DP thymocytes during *T. cruzi* infection, despite their systemic interactions.

Pineal-Thymus Axes and Potential Influence of Thymic Abnormalities during Chagas Disease

The neurohormone melatonin (MEL) is mainly produced by the pineal gland, located beneath the posterior portion of the corpus callosum in the brain. As a neurohormone, it is released into circulation, penetrating all tissues of the body, mostly exerting effects through different pathways. The MEL receptors 1 and 2 (MT1 and MT2, respectively) are expressed by mammalian organs, including the thymus, of various species. Emerging evidence suggests a pivotal role for the pineal gland in modulating the immune response, being their release regulated mainly by NE [74]. Moreover, the relationship between the pineal gland and the immune system is bidirectional, as cytokines, including IFN-γ, IL-1β, and TNF-α also exert influence over MEL synthesis and release [75]. Also, several studies have shown an antiapoptotic role of MEL over thymocytes under stress conditions, while it stimulates proliferation. In this sense, removal of the pineal gland induces a decrease in thymus weight, thymic cell number, whereas the administration of MEL reversed these effects [76], and accordingly, MEL treatment has been proposed as an antioxidant treatment to rejuvenate aging thymus [77]. In addition, MEL is also produced by thymic cells, being their local synthesis regulated by the systemic levels of the hormone [78, 79].

Interestingly, systemic MEL levels were found increased in *T. cruzi*-infected rats, although such an increase was not sufficient to counteract the thymic atrophy induced by the infection [80]. Nevertheless, treatments with exogenous MEL in *T. cruzi*-infected animals seem to have beneficial effects over the thymus, decreasing the production of superoxide (O_2^-) and increasing superoxide dismutase activity, a natural antioxidant [81]. Interestingly, MEL treatment during *T. cruzi* infection increased the percentage of DP thymocytes [80]. Since MEL can inhibit GR signaling in thymocytes [82], the protective effect of MEL on thymic atrophy is likely due in part to this phenomenon.

Final Remarks

Studies conducted in animal models of acute Chagas disease have revealed a marked thymus atrophy, primarily attributed to thymocyte apoptosis, along with diverse functional abnormalities, including the anomalous exit of potentially autoreactive T-cells. Evidently, in this context, the CNS influences thymus physiology through an intricate network achieved by NE, GC, GH, PRL, MEL, and pro-inflammatory cytokines, with particular emphasis on interleukin-1β, IL-6, IFN-γ, and TNF-α (Fig. 3). This prompts the question of whether thymic alterations are simply consequences of systemic neuroendocrine imbalance driven mainly by the SNS or HPA axis, but also PRL/GH axes, alongside with intrathymic circuits influenced by locally produced neuroendocrine ligands, or if they represent a compensatory mechanism to cope with local inflammation and to avoid tolerance to parasite antigens. Likewise, whether thymic products can in any way (i.e., soluble molecules or by axonal retrograde transport) influence brain functions remain an important gap in our knowledge.

Finally, another relevant question arises as to whether observations made in the thymus of *T. cruzi*-infected rodents can be extrapolated to humans and if they hold relevance in the development of Chagas cardiomyopathy. Despite these uncertainties, it is evident that the precise control exerted by the brain over the thymus is markedly disrupted throughout the course of infection. The fact that DN and DP T-cells with an activated phenotype can be tracked in the blood of humans with chronic Chagas disease and also in the heart of infected mice [16], raise new questions about the relevance of these populations in the pathogenesis of Chagas disease and their possible link with thymic alterations and the brain/thymus connectivity. Future studies may highlight the mechanisms underlying these abnormalities and their link with CCC, which may contribute to the design of innovative strategies to control Chagas disease pathology.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

W.S. and A.R.P. conceived the original idea; F.B.G. wrote the manuscript and made the figures with support from W.S. and A.R.P. All authors provided critical feedback and helped shape the manuscript.

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