

Title:

Thymic atrophy during infection as a host response to avoid tolerance to persistent pathogens

1. Abstract

The immune system consists in part of a functionally competent T-cell repertoire that is reactive to foreign antigens but tolerant to self-antigens. The repertoire of T cells is primarily formed in the thymus through positive and negative selection of developing thymocytes that are critical for establishing central tolerance. One of the features of the thymus is to sense stress hormones produced in pathophysiological conditions. Increased levels of these hormones are associated with infections and are able to induce thymic atrophy. We have shown that in acute *Trypanosoma cruzi* infections, the atrophic thymus is a consequence of increased thymocyte apoptosis and premature export of immature thymocytes to secondary lymph nodes. This atrophy does not necessarily result in dysfunction of the thymus since the organ microarchitecture is preserved and maintains negative selection, thus avoiding the development of tolerance to the pathogen during the establishment of protective immunity. However, in chronic infections, the dissemination of invading pathogens able to target the thymus interferes with T cell differentiation, generating T cells that are tolerant to pathogen-specific antigens. In what follows we propose to describe what is known about thymic atrophy induced by infectious pathogens in the context of host-pathogen interactions.

26 **2. The stress hormone circuit as a systemic host sensor for pathogen infections**

27 Infectious pathogens interfere with host physiological homeostasis at several levels.

28 One of the first systemic sensors that identify the danger of invasion is the stress system
29 consisting of a number of responses involving all three regulatory systems, neural,
30 endocrine and immune [1]. In most vertebrates the acute short-term stress signals are
31 responsible for inducing host responses that enhance innate defense mechanisms [2].
32 However, when stress signals persist chronically the host immune response can be
33 suppressed, a scenario that favors the infection [3, 4]. Chronic stress is known to cause a
34 shift from T helper 1-mediated cellular immunity towards T helper 2-mediated humoral
35 immunity, and this can influence the course of an infection and the susceptibility to
36 microorganisms [5, 6].

37 In general, stress signals result from release of neurotransmitters, hormones and
38 cytokines into the circulation, or locally within tissues [7]. The major mediators of
39 stress effects are norepinephrine and epinephrine, which are released by the sympathetic
40 nervous system, and corticotropin-releasing hormone, adrenocorticotropin (ACTH), and
41 cortisol, which make up the hypothalamic-pituitary-adrenal (HPA) axis [8, 9]. All
42 vertebrate cells have receptors for one or more of these factors, allowing them to signal
43 threats from pathogens in response to changes induced by infection [5].

44 Critical synergistic interactions occur between cytokines and stress hormones in
45 inducing the effects of the stress circuits on the immune system [7]. For example, acute
46 psychological stress has been shown to increase circulating IL-1 β levels in both humans
47 and rodents [10, 11] and stress hormones are able to induce similar biological responses
48 to those produced by inflammatory cytokines. This is well demonstrated in the case of
49 glucocorticoids, whose levels increase in response to a variety of infections [5, 11-13].

50 It has been shown in various systems that glucocorticoids synergize with IL-1, IL-6 and
51 other cytokines such as IL-2, IFN- β , granulocyte colony-stimulating factor, granulocyte
52 macrophage colony-stimulating factor, and oncostatin M in inducing acute phase
53 proteins [14]. Some of the interactions between glucocorticoids and cytokines may
54 involve glucocorticoid induced downregulation of cytokine receptors on target cells, as
55 shown by decreased cytokine binding or cytokine receptor mRNA levels[7]. For
56 example, there are reports that glucocorticoids inhibit the expression of IL-2 and its
57 receptor (IL-2R) in leukocytes [15]. This effect accounts for the important role of
58 endogenous glucocorticoids in preventing ongoing immune responses from reaching
59 damaging levels. These observations point to the existence of a negative feedback loop
60 between the immune system and the HPA axis, such that proinflammatory mediators
61 arising in an ongoing immune response stimulate the HPA axis, which in turn results in
62 the secretion of corticosterone and prevents the immune response from reaching level
63 that could damage the host [3, 16].

64 In infections caused by *T. cruzi* parasite, the causative agent of Chagas disease, the
65 inflammatory syndrome induced by TNF- α in the acute phase of infection activates the
66 hypothalamus-pituitary-adrenal (HPA) axis leading to release of corticosterone, and the
67 latter appears to be responsible for changes in both lymphoid and non-lymphoid
68 compartments of the thymus, and for disease outcomes [17, 18]. The protective effects
69 of immunosuppression by endogenous glucocorticoids also have an impact on the
70 thymus, the central lymphoid organ controlling the formation of T cells, which
71 constitute the peripheral host immunological repertoire [19].

72

73 **3. Sensing of glucocorticoids leads to atrophy of the thymus**

74 The thymus is the primary lymphoid organ, located in the thoracic cavity, where T cells
75 are continuously generated and released to the periphery [20]. The complex
76 developmental process responsible for generating T cells within the thymus depends on
77 signals from stromal cells that direct the maturation, clonal expansion and selection of T
78 cell precursors [21]. Defects in the thymic microenvironment can prevent the
79 development of the adaptive immune system, cause severe immunodeficiencies and
80 inhibit immunological surveillance, which is essential for recognizing and removing
81 pathogens and malignant cells in vertebrates [22].

82 All T cell lineages are derived from hematopoietic precursor cells recruited from the
83 blood [23, 24]. The recruited cells have a triple negative CD3⁻CD4⁻CD8⁻ phenotype
84 and penetrate the vascularized microenvironment of the thymus at the boundary
85 between the two major thymic compartments, the cortex and medulla. Early T lineage
86 progenitors enter the thymus and form cell-to-cell contacts with cortical thymic
87 epithelial cells. The engagement between Notch1 receptors on progenitor T cells and
88 their ligand Delta-4-like, expressed by thymic epithelial cells, result in proliferation of
89 the cells and their commitment to the T cell lineage [20]. In subsequent developmental
90 steps, these cells expose both CD4 and CD8 antigens to the clones of antigen-specific T
91 cell receptors (TCR) generated by somatic rearrangement of the variable germinal TCR
92 genes. At this stage the maturing T cells are referred to as double-positive (DP)
93 thymocytes, and these cells must be able to bind self major histocompatibility complex
94 (MHC) molecules, otherwise they die within the first 4 days after the appearance of T
95 cell receptors [25, 26].

96 In this scenario, the initial selection process in the thymus will only retain those T cells
97 that bear an $\alpha\beta$ TCR that is able to recognize self MHC. This process is referred as
98 positive selection and occurs when the T cells bind cortical epithelial cells expressing

99 Class I or Class II MHC plus self-peptides with sufficiently high affinity to receive a
100 survival signal. The maturing cells that are not positively selected are committed to
101 “death by neglect” [27]. The remained thymocytes undergo in the thymic medulla a
102 second selection process known as negative selection, which leads to programmed cell
103 death of T cell clones that by chance express T cell receptors (TCRs) with above-
104 threshold affinities for self-peptide/MHC complexes [25, 28]. Thus, thymocytes capable
105 of generating autoimmune responses are removed and only T cells able to recognize
106 non-self antigens in the context of self MHC molecules are released into the periphery
107 [29]. Upon maturation, thymocytes that express a TCR restricted to class I MHC
108 molecules have the $CD4^-CD8^+$ phenotype while those with an MHC class II-restricted
109 TCR specificity become $CD4^+CD8^-$ cells [27]. After a time required for maturation,
110 these naïve single-positive (SP) T cells exit the thymic medulla and migrate to the
111 periphery in a process that requires signaling via sphingosine 1-phosphate receptor type
112 1 [26, 30].

113 Thymic homeostasis can be severely affected by infection. It has been shown that
114 several infectious pathogens (bacteria, virus, parasites and fungi) can cause atrophy of
115 the organ [31, 32]. It is not completely understood how this atrophy occurs and the
116 mechanism may vary. In most infectious diseases causing thymic atrophy, the major
117 biological event associated with thymocyte loss is cell death by apoptosis [32]. This is
118 seen for example, in experimental models of *Trypanosoma cruzi* infection [33], and
119 malaria in rodents caused by *Plasmodium berghei* [34]. Most of the cells that die are
120 $CD4^+CD8^+$ thymocytes, but other sub-types such as double-negative (DN) T cells and
121 SP cells are also reduced in number. Glucocorticoid hormones appear to be responsible
122 for the thymus atrophy and thymocyte death during parasitic infections. In acute
123 *Trypanosoma cruzi* infections, atrophy is associated with a rise in serum glucocorticoid

124 levels and activation of thymocyte caspases-8 and -9, which promote cell death by
125 apoptosis [33]. Elevated levels of serum glucocorticoids have also been found in other
126 infections: experimentally-induced malaria, African trypanosomiasis, also known as
127 sleeping sickness, toxoplasmosis and leishmaniasis, parasitic diseases caused
128 respectively by the protozoan *Toxoplasma gondii* and *Leishmania* parasites, as well as
129 schistosomiasis, a parasitic disease caused by several species of trematodes belonging to
130 the genus *Schistosoma* [35].

131 **4. Thymic atrophy during *Trypanosoma* infections does not interfere with negative** 132 **selection**

133 The primary event in thymic atrophy is a severe reduction in thymic cell numbers,
134 which is reflected in an equally dramatic reduction in thymic output, potentially
135 reducing peripheral T cell numbers [31]. Although the induction of thymic atrophy is
136 considered a virulence trait shared by many pathogens, until recently it was not clear
137 whether intrathymic negative selection was affected by the alterations in the thymic
138 microenvironment. During thymopoiesis, clonal deletion appears to occur normally late
139 in thymocyte development at the transition from DP to SP cells, and happens at the
140 junction between cortex and medulla [36, 37]. Thymic metallophilic macrophages have
141 been shown to be involved in thymocyte maturation at this interface. Like cortical
142 macrophages and medullary interdigitating cells, metallophilic macrophages contain
143 large endocytic compartments devoted to processing and presentation of antigens by
144 MHC class II molecules [38, 39]. Interestingly, we have shown that the distribution of
145 these metallophilic macrophages is altered by infection: their number increases, and
146 while some remain in the cortico-medullary region many more are spread throughout
147 the cortex. The thymic distribution of these cells may indicate that clonal selection
148 switches from the cortico-medullary junction to the cortex [40].

149 It has been consistently shown that thymopoiesis requires sustained interactions
150 between thymic epithelial cells (TEC) and thymocytes [21]. When the thymic
151 architecture is disturbed, thymic function is severely compromised, since the pattern of
152 expression of autoantigens by TECs is altered [41, 42]. In an experimental murine
153 model of *T. cruzi* infection, we detected similar expression levels of Aire and highly
154 selective tissue-restricted antigens in whole thymuses of *T. cruzi*-infected mice and non-
155 infected control mice [40]. These findings suggest that the expression of peripheral
156 antigens in the infected thymuses is sufficient to modulate the induction of tolerance by
157 negative selection.

158 In *T. cruzi*-infected mice, thymic atrophy becomes apparent after a rise in the number of
159 apoptotic intrathymic DP T cells caused at least in part by increased systemic
160 glucocorticoid levels [33, 43]. We found that expression of Bim, a pro-apoptotic factor
161 essential for negative selection [44], was maintained while the number of DPs declined
162 [40]. Furthermore, using the OTII TCR transgenic system specific for a CD4⁺-epitope
163 of the chicken ovalbumin (OVA) protein, in which all the T cells are OVA-specific, we
164 observed that apoptosis of TCR-stimulated immature thymocytes was promoted when
165 the cognate OVA peptide was *in vivo* administered to acutely infected mice undergoing
166 thymic atrophy [40]. These data provide evidence that DP T cells can be negatively
167 selected during thymic atrophy, suggesting that negative selection operates normally
168 during infection. This is consistent with the finding that mature single-positive CD4⁺
169 and CD8⁺ T cells within the thymus do not harbor forbidden TCR genes, unlike their
170 DP counterparts, indicating that immature thymocytes bearing auto-specific TCR
171 continue to be negatively selected in the thymus [40, 45].

172

173

174

175 **5. Chronic persistent infections of pathogens targeting the thymus subvert central**
176 **tolerance**

177 Thymic atrophy in pathogen infections has been explained as a host response aimed at
178 controlling thymopoiesis in order to prevent tolerance to the colonizing pathogen [31,
179 46, 47]. The severe loss of thymic cell numbers reduces thymic output [46]. However
180 pathogens such as *M. avium* that target the thymus are able to induce the production of
181 new T cells to the host repertoire, but these T cells are unable to mount a protective
182 response against the pathogen in the periphery [48] . In this study, it was shown that T
183 cells from *M. avium* infected animals are deficient in producing IFN- γ in response to
184 mycobacterial antigens, and are therefore unable to control bacterial growth [48].

185 Interestingly, *M. avium* was found to be present inside macrophages and dendritic cells
186 (DCs) in the medulla as well as in the cortico-medullary thymic regions [48]. Since DCs
187 are considered key players in the negative selection of specific-T cell [49], presentation
188 of mycobacterial antigens by thymic DCs would be able to induce clonal deletion of *M.*
189 *avium*-specific T cells. This event is thought to be responsible for the observed host
190 tolerance to *M. avium*[48] . However, since there is also a pathway in the thymus
191 leading to the development of regulatory T cell recognizing specific antigens with high
192 affinity [50, 51], the generation of mycobacteria-specific regulatory T cells could not be
193 discarded in the tolerance to *M. avium*.

194 Furthermore, these studies show that infection of the thymus by mycobacteria targets T
195 cell recirculation from the periphery to the thymus, which guarantees that the immune
196 system can respond to thymic infection [33]. The *M. avium*-infected thymus increases
197 the production of chemokines, such as CXCL9 and CXCL10, known to recruit CXCR3⁺
198 peripheral T cells involved in the control of bacterial infections [52]. As these cells

199 recycle into the thymus, they may become susceptible to clonal deletion through
200 negative selection. These findings together indicate that *M. avium* infection of the
201 thymus promotes the loss of *M. avium*-specific T cells, resulting in host tolerance to the
202 pathogen and favoring chronic infection [48, 52]. This process is an important aspect of
203 the host-pathogen interplay in infections that target the thymus.

204

205 **6. Concluding Remarks**

206 Infectious thymic atrophy is a consequence of host colonization by bacteria, viruses,
207 and parasites and is considered a common response to pathogens. Various hypotheses
208 have been proposed to explain this atrophy, including increased apoptosis of developing
209 thymocytes, premature egress of immature thymocytes bearing undesired TCR
210 specificities, and thymic hibernation inhibiting the thymocyte selection process and
211 preventing the establishment of central tolerance to pathogen antigens that may access
212 the thymic tissues. The latter hypothesis is relevant to host-pathogen interplay in many
213 chronic infections. It has gained strength from the recent demonstration of pathogen-
214 induced tolerance in thymic infections by *M. avium*. Those studies have demonstrated
215 that pathogen-derived antigens present in the thymus of infected mice seem to interfere
216 with the capacity of pathogen-specific activated T cells to respond to the invading
217 organism. This is highly relevant to our understanding of the development of host
218 immunity during persistent pathogen infections. The presence of pathogen antigens in
219 the thymus may in fact promote the recirculation of activated T cells from the periphery
220 to the thymus resulting either in clonal deletion of pathogen-specific T cells or possible
221 in the generation of pathogen-specific regulatory T cells that induce tolerance to a
222 persistent infection. These concepts are important for our understanding of the

223 establishment of T cell protective immunity in the host and its ability to control chronic
224 persistent pathogen infections.

225

226 **7. Acknowledgments**

227 This work was supported by grants from Conselho Nacional de Desenvolvimento
228 Científico e Tecnológico do Brasil (CNPq), Coordenação de Aperfeiçoamento de
229 Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado do
230 Rio de Janeiro (FAPERJ).

231

232

233 **8. Figure Legend**

234 **Figure 1. Stress hormones induced during infection promote atrophy of the**
235 **thymus.** The hypothalamic-pituitary-adrenal (HPA) axis originates in the brain and is
236 involved in recognizing signal stressors from infections, such as IL1, IL6 and TNF- α .
237 After a pathogen infection, the hypothalamus releases corticotropin-releasing factor
238 (CRF) to the pituitary gland, which is rich in CRF type 1 receptors (CRFR-1). In
239 response, the pituitary releases adrenocorticotrophic hormone (ACTH) into the
240 bloodstream. This hormone targets the adrenal glands, located atop the kidneys. These
241 glands then release the glucocorticoid (GC) hormone cortisol, which causes thymic
242 atrophy. In the atrophic thymus there is a reduced output of recent-thymic emigrants. T
243 cells to the periphery, which guarantees that normal thymopoiesis is accompanied by
244 negative selection of T cells bearing undesired TCR specificities. However in chronic
245 infections some pathogens can colonize the thymus persistently; as a result their
246 antigens are presented in the context of negative selection and can induce central
247 tolerance to the corresponding organism.

248

249 9. References

- 250 1. Besedovsky, H.O; del Rey, A. Immune-neuro-endocrine interactions: facts and
251 hypotheses. *Endocrine Review* **1996**, 17, 64-102.
- 252 2. Kanczkowski, W.; Zacharowski, K.; Bornstein, S.R. Role of toll-like receptors
253 and inflammation in adrenal gland insufficiency. *Neuroimmunomodulation*
254 **2010**, 17, 180-183.
- 255 3. Nicolaides, N.C.; Galata, Z.; Kino, T.; Chrousos, G.P.; Charmandari, E. The
256 human glucocorticoid receptor: molecular basis of biologic function. *Steroids*,
257 **2010**, 75, 1-12.
- 258 4. Baker, R.W.; Walker, B.R.; Shaw, R.J.; Honour, J.W.; Jessop, D.S.; Lightman,
259 S.L.; Zumla, A.; Rook, G.A. Increased cortisol: cortisone ratio in acute
260 pulmonary tuberculosis. *American Journal of Respiratory and Critical Care*
261 *Medicine* **2000**, 162, 1641-1647.
- 262 5. Martino, M.; Rocchi, G.; Escelsior, E.; Fornaro, M. Immunomodulation
263 Mechanism of Antidepressants: Interactions between Serotonin/Norepinephrine
264 Balance and Th1/Th2 Balance. *Current Neuropsychopharmacology* **2012**, 10, 97-123.
- 265 6. Elenkov, I.J.; Chrousos, G.P. Stress Hormones, Th1/Th2 patterns, Pro/Anti-
266 inflammatory Cytokines and Susceptibility to Disease. *Trends Endocrinology*
267 *and Metabolism* **1999**, 10, 359-368.
- 268 7. Turnbull, A.V.; Rivier, C.L. Regulation of the hypothalamic-pituitary-adrenal
269 axis by cytokines: actions and mechanisms of action. *Physiological Reviews*
270 **1999**, 79, 1-71.
- 271 8. Elenkov, I.J. Neurohormonal-cytokine interactions: implications for
272 inflammation, common human diseases and well-being. *Neurochemistry*
273 *International* **2008**, 52, 40-51.
- 274 9. Levite, M. Neurotransmitters activate T-cells and elicit crucial functions via
275 neurotransmitter receptors. *Current Opinion in Pharmacology* **2008**, 8, 460-471.
- 276 10. Speaker, K.J.; Fleshner, M. Interleukin-1 beta: a potential link between stress and
277 the development of visceral obesity. *BMC Physiology* **2012**, 12, 8-23.
- 278 11. Rook, G.A. Glucocorticoids and immune function. *Baillière's best practice and*
279 *research. Clinical endocrinology and metabolism* **1999**, 13, 567-581.
- 280 12. Rook, G.A.; Onyebujoh, P.; Stanford, J.L. TH1/TH2 switching and loss of
281 CD4+ T cells in chronic infections: an immunoendocrinological hypothesis not
282 exclusive to HIV. *Immunology Today* **1993**, 14, 568-569.

- 283 13. Kelestimur, F. The endocrinology of adrenal tuberculosis: the effects of
284 tuberculosis on the hypothalamo-pituitary-adrenal axis and adrenocortical
285 function. *Journal of Endocrinological Investigation* **2004**, 27, 380-386.
- 286 14. Hernandez-Rodriguez, J.; Segarra, M.; Vilardell, C.; Sánchez, M.; García-
287 Martínez, A.; Esteban, M.J.; Queral, C.; Grau, J.M.; Urbano-Márquez, A.;
288 Palacín, A.; Colomer, D.; Cid, M.C. Tissue production of pro-inflammatory
289 cytokines (IL-1beta, TNFalpha and IL-6) correlates with the intensity of the
290 systemic inflammatory response and with corticosteroid requirements in giant-
291 cell arteritis. *Rheumatology (Oxford)* **2004**, 43, 294-301.
- 292 15. Paliogianni, F.; Ahuja, S.S.; Balow, J.P.; Balow, J.E.; Boumpas, D.T. Novel
293 mechanism for inhibition of human T cells by glucocorticoids. Glucocorticoids
294 inhibit signal transduction through IL-2 receptor. *Journal of Immunology* **1993**,
295 151, 4081-4089.
- 296 16. Karin, M; Chang, L. AP-1--glucocorticoid receptor crosstalk taken to a higher
297 level. *Journal Endocrinology* **2001**, 169, 447-451.
- 298 17. Roggero, E.; Pérez AR, Bottasso OA, Besedovsky HO, Del Rey A.
299 Neuroendocrine-immunology of experimental Chagas' disease. *Annals of the*
300 *New York Academy of Sciences* **2009**, 1153, 264-271.
- 301 18. Villar, S.R., et al., Tumor Necrosis Factor-alpha Regulates Glucocorticoid
302 Synthesis in the Adrenal Glands of Trypanosoma cruzi Acutely-Infected Mice.
303 The Role of TNF-R1 *PLoS One* **2013**, 8, e63814.
- 304 19. Perez, A.R.; Berbert, L.R.; Lepletier, A.; Revelli, S.; Bottasso, O.; Silva-
305 Barbosa, S.D.; Savino, W. TNF-alpha is involved in the abnormal thymocyte
306 migration during experimental Trypanosoma cruzi infection and favors the
307 export of immature cells. *PLoS One* **2012**, 7, e34360.
- 308 20. Thompson, P.K.; Zuniga-Pflucker, J.C. On becoming a T cell, a convergence of
309 factors kick it up a Notch along the way. *Seminars in Immunology* **2011**, 23,
310 350-359.
- 311 21. Anderson, G.; Takahama, Y. Thymic epithelial cells: working class heroes for T
312 cell development and repertoire selection. *Trends in Immunology* **2012**, 33, 256-
313 263.
- 314 22. Poliani, P.L.; Vermi, W.; Facchetti, F. Thymus microenvironment in human
315 primary immunodeficiency diseases. *Current Opinion in Allergy and Clinical*
316 *Immunology* **2009**, 9, 489-495.
- 317 23. Zlotoff, D.A.; Sambandam, A.; Logan, T.D.; Bell, J.J.; Schwarz, B.A.;
318 Bhandoola, A. CCR7 and CCR9 together recruit hematopoietic progenitors to
319 the adult thymus. *Blood* **2010**, 115, 1897-1905.

- 320 24. Gwin, K.A.; Shapiro, M.B.; Dolence, J.J.; Huang, Z.L.; Medina, K.L. Hoxa9
321 and flt3 signaling synergistically regulate an early checkpoint in lymphopoiesis.
322 *Journal of Immunology* **2013**, 191, 745-754.
- 323 25. Gascoigne, N.R.; Palmer, E. Signaling in thymic selection. *Current Opinion in*
324 *Immunology* **2011**, 23, 207-212.
- 325 26. Mendes-da-Cruz, D.A.; Stimamiglio, M.A.; Muñoz, J.J.; Alfaro, D.; Terra-
326 Granado, E.; Garcia-Ceca, J.; Alonso-Colmenar, L.M.; Savino, W.; Zapata, A.G.
327 Developing T-cell migration: role of semaphorins and ephrins. *FASEB Journal*
328 **2012**, 26, 4390-4399.
- 329 27. Lo, W.L.; Allen, P.M. Self-awareness: how self-peptide/MHC complexes are
330 essential in the development of T cells. *Molecular Immunology* **2013**, 55, 186-
331 189.
- 332 28. Currie, J.; Castro, M.; Lythe, G.; Palmer, E.; Molina-París, C. A stochastic T cell
333 response criterion. *Journal of the Royal Society Interface* **2012**, 9, 2856-2870.
- 334 29. Williams, C.B.; Engle, D.L.; Kersh, G.J.; Michael White, J.; Allen, P.M. A
335 kinetic threshold between negative and positive selection based on the longevity
336 of the T cell receptor-ligand complex. *Journal of Experimental Medicine* **1999**,
337 189, 1531-1544.
- 338 30. Love, P.E.; Bhandoola, A. Signal integration and crosstalk during thymocyte
339 migration and emigration. *Nature Reviews Immunology* **2011**, 11, 469-477.
- 340 31. Savino, W. The thymus is a common target organ in infectious diseases. *PLoS*
341 *Pathogen* **2006**, 2, e62.
- 342 32. de Meis, J.; Farias-de-Oliveira, D.A.; Nunes-Panzenhagen, P.H.; Maran, N.;
343 Villa-Verde, D.M.; Morrot, A.; Savino, W. Thymus atrophy and double-positive
344 escape are common features in infectious diseases. *Journal of Parasitology*
345 *Reserch* **2012**, 2012, 574020.
- 346 33. Lepletier, A.; de Frias Carvalho, V.; Morrot, A.; Savino, W. Thymic atrophy in
347 acute experimental Chagas disease is associated with an imbalance of stress
348 hormones. *Annals of the New York Academy of Sciences* **2012**, 1262, 45-50.
- 349 34. Andrade, C.F.; Gameiro, J.; Nagib, P.R.; Carvalho, B.O.; Talaisys, R.L.; Costa,
350 F.T.; Verinaud, L. Thymic alterations in Plasmodium berghei-infected mice.
351 *Cellular Immunology* **2008**, 253, 1-4.
- 352 35. Wellhausen, S.R.; Boros, D.L. Atrophy of the thymic cortex in mice with
353 granulomatous schistosomiasis mansoni. *Infection and Immunity* **1982**, 35,
354 1063-1069.
- 355 36. Hogquist, K.A.; Baldwin, T.A.; Jameson, S.C. Central tolerance: learning self-
356 control in the thymus. *Nature Reviews Immunology* **2005**, 5, 772-782.

- 357 37. Ladi, E.; Yin, X.; Chtanova, T.; Robey, E.A. Thymic microenvironments for T
358 cell differentiation and selection. *Nat Immunol* **2006**, *7*(4): p. 338-43.
- 359 38. Milicevic, N.M.; Milicevic, Z.J. Enzyme-histochemical characterization of
360 macrophages in the rat thymus, with special reference to metallophilic cells of
361 the corticomedullary zone. *Journal of Leukocyte Biology* 1984, *36*, 761-769.
- 362 39. Milicevic, N.M.; Milicevic, Z.J. Metallophilic macrophages of the rodent
363 thymus. *Progress in Histochemistry and Cytochemistry* **2013**, *48*, 1-46.
- 364 40. Morrot, A.; Terra-Granado, E.; Pérez, A.R.; Silva-Barbosa, S.D.; Milićević,
365 N.M.; Farias-de-Oliveira, D.A.; Berbert, L.R.; de Meis, J.; Takiya, C.M.;
366 Beloscar, J.; Wang, X.; Kont, V.; Peterson, P.; Bottasso, O.; Savino, W.
367 Chagasic thymic atrophy does not affect negative selection but results in the
368 export of activated CD4+CD8+ T cells in severe forms of human disease. *PLoS*
369 *Neglected Tropical Diseases* **2011**, *5*, e1268.
- 370 41. Xiao, S.; Manley, N.R. Impaired thymic selection and abnormal antigen-specific
371 T cell responses in Foxn1(Delta/Delta) mutant mice. *PLoS One* **2010**, *5*, e15396.
- 372 42. Tykocinski, L.O., Sinemus, A.; Kyewski, B. The thymus medulla slowly yields
373 its secrets. *Ann N Y Acad Sci*, **2008**, 1143, 105-122.
- 374 43. Farias-de-Oliveira, D.A.; Villa-Verde, D.M.; Nunes Panzenhagen, P.H.; Silva
375 dos Santos, D.; Berbert, L.R.; Savino, W.; de Meis, J. Caspase-8 and caspase-9
376 mediate thymocyte apoptosis in Trypanosoma cruzi acutely infected mice.
377 *Journal of Leukocyte Biology* **2013**, *93*, 227-234.
- 378 44. Bouillet, P.; Purton, J.F.; Godfrey, D.I.; Zhang, L.C.; Coultas, L.; Puthalakath,
379 H.; Pellegrini, M.; Cory, S.; Adams, J.M.; Strasser, A. BH3-only Bcl-2 family
380 member Bim is required for apoptosis of autoreactive thymocytes. *Nature* **2002**,
381 *415*, 922-926.
- 382 45. Mendes-da-Cruz, D.A.; de Meis, J.; Cotta-de-Almeida, V.; Savino, W.
383 Experimental Trypanosoma cruzi infection alters the shaping of the central and
384 peripheral T-cell repertoire. *Microbes and Infection* **2003**, *5*, 825-832.
- 385 46. Sodora, D.L.; Milush, J.M.; Ware, F.; Wozniakowski, A.; Montgomery, L.;
386 McClure, H.M.; Lackner, A.A.; Marthas, M.; Hirsch, V.; Johnson, R.P.; Douek,
387 D.C.; Koup, R.A. Decreased levels of recent thymic emigrants in peripheral
388 blood of simian immunodeficiency virus-infected macaques correlate with
389 alterations within the thymus. *Journal of Virology* **2002**, *76*, 9981-9990.
- 390 47. Ross, E.A.; Coughlan, R.E.; Flores-Langarica, A.; Lax, S.; Nicholson, J.;
391 Desanti, G.E.; Marshall, J.L.; Bobat, S.; Hitchcock, J.; White, A.; Jenkinson,
392 W.E.; Khan, M.; Henderson, I.R.; Lavery, G.G.; Buckley, C.D.; Anderson, G.;
393 Cunningham, A.F. Thymic function is maintained during Salmonella-induced
394 atrophy and recovery. *Journal of Immunology* **2012**, *189*, 4266-4274.

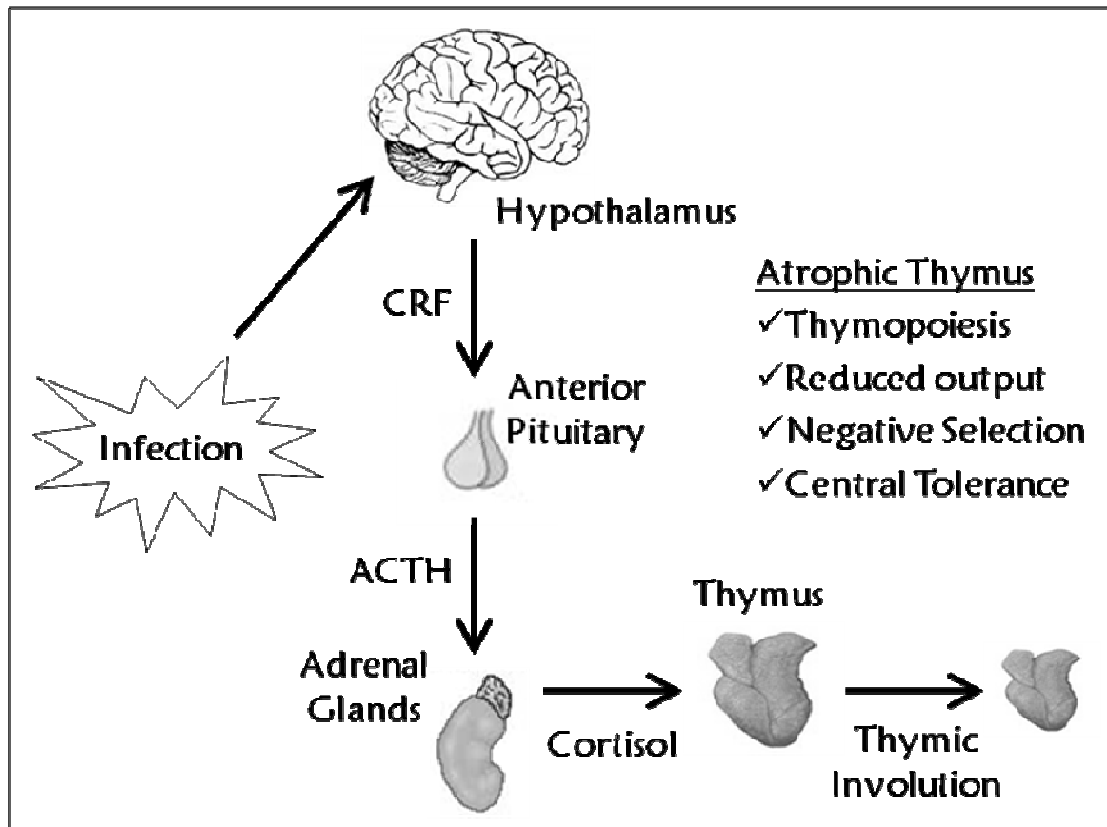
- 395 48. Nobrega, C.; Roque, S.; Nunes-Alves, C.; Coelho, A.; Medeiros, I.; Castro,
396 A.G.; Appelberg, R.; Correia-Neves, M. Dissemination of mycobacteria to the
397 thymus renders newly generated T cells tolerant to the invading pathogen.
398 *Journal of Immunology* **2010**, 184, 351-358.
- 399 49. Goldschneider, I.; Cone, R.E. A central role for peripheral dendritic cells in the
400 induction of acquired thymic tolerance. *Trends in Immunology* **2003**, 24, 77-81.
- 401 50. Proietto, A.I.; van Dommelen, S.; Zhou, P.; Rizzitelli, A.; D'Amico, A.; Steptoe,
402 R.J.; Naik, S.H.; Lahoud, M.H.; Liu, Y.; Zheng, P.; Shortman, K.; Wu, L.
403 Dendritic cells in the thymus contribute to T-regulatory cell induction.
404 *Proceedings of the National Academy of Sciences of the United States of*
405 *America* **2008**, 105, 19869-19874.
- 406 51. Atibalentja, D.F.; Byersdorfer, C.A.; Unanue, E.R. Thymus-blood protein
407 interactions are highly effective in negative selection and regulatory T cell
408 induction. *J Immunol* **2009**, 183, 7909-7918.
- 409 52. Nobrega, C.; Nunes-Alves, C.; Cerqueira-Rodrigues, B.; Roque, S.; Barreira-
410 Silva, P.; Behar, S.M.; Correia-Neves, M. T cells home to the thymus and
411 control infection. *Journal of Immunology*, **2013**, 190, 1646-1658.

412

413

414

415 **Figure 1**



416