Ann. N.Y. Acad. Sci. ISSN 0077-8923

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Issue: *Neuroimmunomodulation in Health and Disease*

Extrathymic CD4$^+$CD8$^+$ lymphocytes in Chagas disease: possible relationship with an immunoendocrine imbalance

Ana R. Pérez,[1] Alexandre Morrot,[2] Luiz R. Berbert,[3] Eugenia Terra-Granado,[3,4] and Wilson Savino[3]

[1]Institute of Immunology, Faculty of Medical Sciences, National University of Rosario, Rosario, Argentina. [2]Department of Immunology, Microbiology Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. [3]Laboratory on Thymus Research, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil. [4]Pediatric Hematology and Oncology Program, Research Center, National Cancer Institute, Rio de Janeiro, Brazil

Address for correspondence: Ana Rosa Pérez, Institute of Immunology, Faculty of Medical Sciences, National University of Rosario, Santa Fe 3100 (2000), Rosario, Argentina. perez_anarosa@yahoo.com.ar; perez.ana@conicet.gov.ar

Double-positive (DP) CD4$^+$CD8$^+$ T cells normally represent a thymic subpopulation that is developed in the thymus as a precursor of CD4$^+$ or CD8$^+$ single-positive T cells. Recent evidence has shown that DP cells with an activated phenotype can be tracked in secondary lymph organs. The detection of an activated DP population in the periphery, a population that expresses T cell receptors unselected during thymic negative selection in murine models of *Trypanosoma cruzi* infection and in humans with Chagas disease, raise new questions about the relevance of this population in the pathogenesis of this major parasitic disease and its possible link with immunoendocrine alterations.

Keywords: CD4$^+$CD8$^+$ double-positive CD4$^+$CD8$^+$ T cells; thymus; Chagas disease; *Trypanosoma cruzi* infection; cortisol; dehydroepiandrosterone

Double-positive CD4$^+$CD8$^+$ T cells: from physiology to pathology

The simultaneous expression of CD4 and CD8 in T cells was, until some years ago, generally considered exclusive for T cells in the thymus. However, T cells expressing both CD4 and CD8 coreceptors have been described in healthy individuals, as well as in pathological conditions such as infectious diseases, autoimmune diseases, chronic inflammatory disorders, and certain lymphoblastic diseases (Table 1).[1–3] Most double-positive (DP) CD4$^+$ CD8$^+$ T cells undergo differentiation to single-positive (SP) CD4$^+$ or CD8$^+$ mature T cells in the thymus. However, the presence of T cells coexpressing both CD4 and CD8 in the periphery raises new questions, such as, What is the origin of these lymphocytes? Do these cells exit from the thymus as immature or mature T cells? Is it possible that during the development of inflammatory responses SP T cells acquire any other cell marker, thus showing a certain degree of plasticity? Do the extrathymic DP cells have specific functions? An additional, more intriguing question is: are DP cells involved in the pathophysiology of autoimmune events occurring in some infectious conditions, particularly in the pathogenesis of Chagas disease?

Before addressing these issues, it is worthwhile to provide a general background on intrathymic DP cell differentiation and T cell exportation to the periphery of the immune system. The thymus is the primary lymphoid organ responsible for the differentiation of T cells. This process involves differential expression of CD4 or CD8 accessory molecules (among others) and rearrangements of T cell receptor (TCR) genes. The most immature thymocytes express neither the TCR complex nor the CD4 or CD8 markers, and for this reason they are called *double-negative cells*, a subset representing nearly 5% of total thymocytes. The process of maturation follows with the acquisition of CD4 and CD8 markers, generating the DP cells, which constitute

doi: 10.1111/j.1749-6632.2012.06627.x

Table 1. Diseases with presence of DP $CD4^+CD8^+$ T cells in periphery

Diseases with ↑% DP	Functional characteristics	Phenotype	Human or animal model	References
Infectious diseases				
Chagas disease	Citotoxic activity ↑INF-γ production	Activated phenotype ↑ expression CD44 / CD69 ↑ expression HLA-DR / VLA-4	Murine and human	27
	↑% *T. cruzi*-specific DP cells Cytotoxic activity ↑INF-γ production	Activated phenotype	Human	28
Malaria			Murine	32
T. evansi infection			Sheep	31
Hepatitits		↑% $CD4^{+high}CD8^{+low}$, $CD4^{+low}CD8^{+high}$, and $CD4^{+high}CD8^{+high}$	Human	33
		Effector/memory T cells		36
HIV	↑%HIV-specific DP cells ↑INF-γ production ↑IL-2 production ↑expression of cytolytic-associated lysosomal-associated membrane protein		Human	37
		↑$CD8^{high}CD4^{low}$	Human	38,39
SIV		↑Memory markers ($CD28CD95CD45RA^{low}$ $CD62L^{low}$) ↓% CCR7	Monkey	40
HTLV-1			Human	41
		↑ $CD45RO^+CD18^+CD54^+$	Human	42
			Human	43
HTLV-1/CMV co-infection			Human	44
HBV		↑ $CD4^{low}CD8^{high}$HLA-DR^+ ↑$CD4^{high}CD8^{low}CD56^+CD57^+$	Human	34
Chlamydia pneumoniae infection			Murine	45
Autoimmune diseases and other noninfectious processes				
Immunosenescence		↑% $CD4^{high}CD8^{low}$, $CD4^{low}CD8^{high}$	Human	46
Myasthenia gravis			Human	47
Sjögren's syndrome			Human	48
Multiple sclerosis			Human	49
Sclerosis	↑% DP in skin ↑production of IL-4		Human	50
Thyroiditis	DP cells in thyroid		Human	29
Rheumatoid arthritis	DP cells in synovial fluids		Human	30

Continued

Table 1. *Continued*

Diseases with ↑% DP	Functional characteristics	Phenotype	Human or animal model	References
Myelodysplastic syndromes	↓% DP		Human	51
Leukemia associated with HTLV-1 infection			Human	52
			Human	53
Breast cancer	Cytotoxic activity ↑production of IL-5 and IL-13.	Effector/memory activated $CD8^+$	Human	54
Hodgkin lymphoma		Activated/regulatory phenotype	Human	55
		Expression of $CD3^+$ $CD5^+$ $CD2^+$ $CD7^+$ $CD1a^-$ and TDT	Human	56

75–80% of the whole thymocyte population. At this stage, TCRs are expressed on the cell surface, allowing two crucial events for thymocyte differentiation: positive and the negative selection. Differentiation of α/β TCR-expressing T cells involves an obligatory interaction with self-major histocompatibility complex molecules (MHC) in the thymus. This process, called *positive selection*, not only rescues thymocytes from programmed cell death but also induces their differentiation into mature T cells. Another critical event in thymic development is to prevent maturation of hazardous autoreactive T cells, thus eliminating T cells with self-reactive receptors (*negative selection*). Negative selection allows the establishment of self-tolerance in the T cell repertoire, promoting the apoptosis of T cells that might react against self-proteins. At this stage, immature DP thymocytes become mature SP cells, constituting nearly 20% of thymocytes. The majority of thymocytes die in the thymus, and only a small proportion leaves the organ as recent thymic emigrants (RTEs). Overall, this process is partially controlled by immunoneuroendocrine circuits.[4,5] Of note, the survival of DP cells is negatively influenced by glucocorticoids (GCs) and tumor necrosis factor-alpha (TNF-α).[6–9]

In normal conditions, it is estimated that the thymuses of adult mice export daily only 1% of total thymocytes, representing between 1 and 2 million cells.[10,11] During their journey, thymocytes interact with diverse components of the thymic microenvironment, comprising thymic epithelial cells, macrophages, dendritic cells, fibroblasts, as well as extracellular matrix (ECM) proteins, such as fibronectin or laminin. ECM-mediated interactions can influence the general process of thymocyte maturation, differentiation, and consequent T cell export to the peripheral immune system.

Are DP cells relevant in the pathophysiology of Chagas disease?

Chagas disease, a tropical neglected disease, is caused by the parasite *Trypanosoma cruzi*. Nearly 12 million people are infected in Latin America,[12] and it has spread to nonendemic zones, including the United States, Europe, Asia, and Oceania, and represents a new world health problem, considering that Chagas disease can also be spread by congenital transmission, blood transfusion, or organ transplantation.[13] The clinical manifestations of disease can be highly heterogeneous. After the first contact with the parasite, infected individuals develop an acute phase of disease, which can be asymptomatic or present fever, lymphoadeno, and/or splenomegaly. Following the disappearance of the parasite in the blood, infected individuals can remain asymptomatic for the rest of their lives, a period known as the "indeterminate form of the chronic phase of disease." Between 15 and 30 years after the initial infection, nearly 30% of infected individuals develop chronic chagasic myocarditis, a hallmark of disease. The causes and mechanisms associated with the development and the

establishment of different clinical manifestations of Chagas disease seem complex and remain to be precisely defined. Yet, autoimmune reactions, as well as the consequences of parasite persistence, have already been largely studied.[14,15] It is possible that the indeterminate form of chronic Chagas disease occurs when immunological response of the host against the parasite is more efficient, as during the acute phase, whereas the symptomatic forms seen in the chronic phase seem to occur in patients with hyperergic or inefficient immune responses.[16]

SP T cells play a key role in both the protection against the parasite and in immunopathology.[17–19] For this reason, historically, the study of Chagas disease has mainly focused on the effector immune response directed at the parasite, as well as autoimmune reactions observed against the heart. Nevertheless, the putative involvement of the thymus-derived T lymphocytes in the immunopathology of Chagas disease, as well as the impact of the host's response in the thymus, have not been explored. In this respect, we recently found that RTEs in acutely infected chagasic animals are essentially SP cells, and that their relative and absolute numbers increase progressively with the infection, suggesting an abnormal release of T cells.

During *T. cruzi* infection, the thymus is severely affected by a dysregulated circuit of proinflammatory cytokines and hypothalamus–pituitary–adrenal (HPA)-related hormones.[20,21] Thymus atrophy is commonly observed during murine models of *T. cruzi* acute infection and persists, although to a lower extent, during the chronic phase. Thymic atrophy is essentially secondary to massive depletion of the DP T cell population, at least partially caused by an immunoendocrine imbalance, with enhanced levels of GCs as a result of an exaggerated increase in the levels of inflammatory cytokines.[21,22] In addition to the apoptosis seen in the DP subset, other changes might account for the loss of these cells, such as an increase in their export from the thymus, a decrease in their proliferation rate, and/or a diminution in their numbers secondary to a low recruitment of bone marrow–derived precursors.[23] Among the causes that could influence an increase in the DP cell exportation is the abnormally high intrathymic expression of ECM ligands and receptors.[24]

The first evidence of an aberrant thymic release of DP lymphocytes to the periphery after murine acute *T. cruzi* infection was the observation that DP cells progressively accumulated in peripheral lymphoid organs.[24] Actually, they can also be detected in circulation and in low numbers within the heart, a major target organ in Chagas disease (Fig. 1A). The DP cells seem to be thymus-dependent because their presence in the periphery is largely reduced when the infection is carried out in thymectomized mice. Moreover, studies performed in BALB/c mice showed that some of these extrathymic DP cells carry prohibited V_β segments of the TCR,[24,25] leading to the hypothesis that they have escaped from negative selection, and thus have the potential to be autoreactive.

As seen in Figure 1B, an abnormal increase in DP cell export results in a progressive augmentation in their relative and absolute numbers in both lymph nodes and the spleen of infected animals. In these studies, thymocytes were previously intrathymically labeled with fluorescein isothiocyanate (FITC), and 16 hours later RTEs ($FITC^+$ cells) were tracked in peripheral lymphoid organs.[23] The augmented presence of DP cells in peripheral organs (including the heart) might represent an accelerated recruitment of T cells from thymus as a compensatory mechanism to overcome the anergy/immunosupression described during the acute phase of *T. cruzi* infection.[26]

More recently, we determined that such abnormal extrathymic DP cells bear an activated phenotype, with upregulated expression of the activation markers CD44 and CD69 at levels comparable with activated/peripheral $CD4^+$ or $CD8^+$ SP T cells. Interestingly, CD62L (L-selectin) is expressed normally in RTEs and mature SP T cells; but during acute infection, CD62L expression in DP cells within the thymus is comparable to expression of CD62L on SP cells in the periphery.[27] In fact, upregulation of CD62L, which directs lymphocyte homing to lymph nodes, may favor the exit of DP cells from the thymus of *T. cruzi*–infected individuals.

In addition to the activated status of T cells, peripheral DP cells upregulate TCR expression levels (Fig. 1C). Surface TCR expression level greatly influences T cell antigen sensitivity. This level is rapidly downregulated when T cells are stimulated with strong TCR agonists, as engaged TCRs are internalized and degraded. The increased expression of TCR on peripheral DP cells may help promote sustained antigenic signaling in the

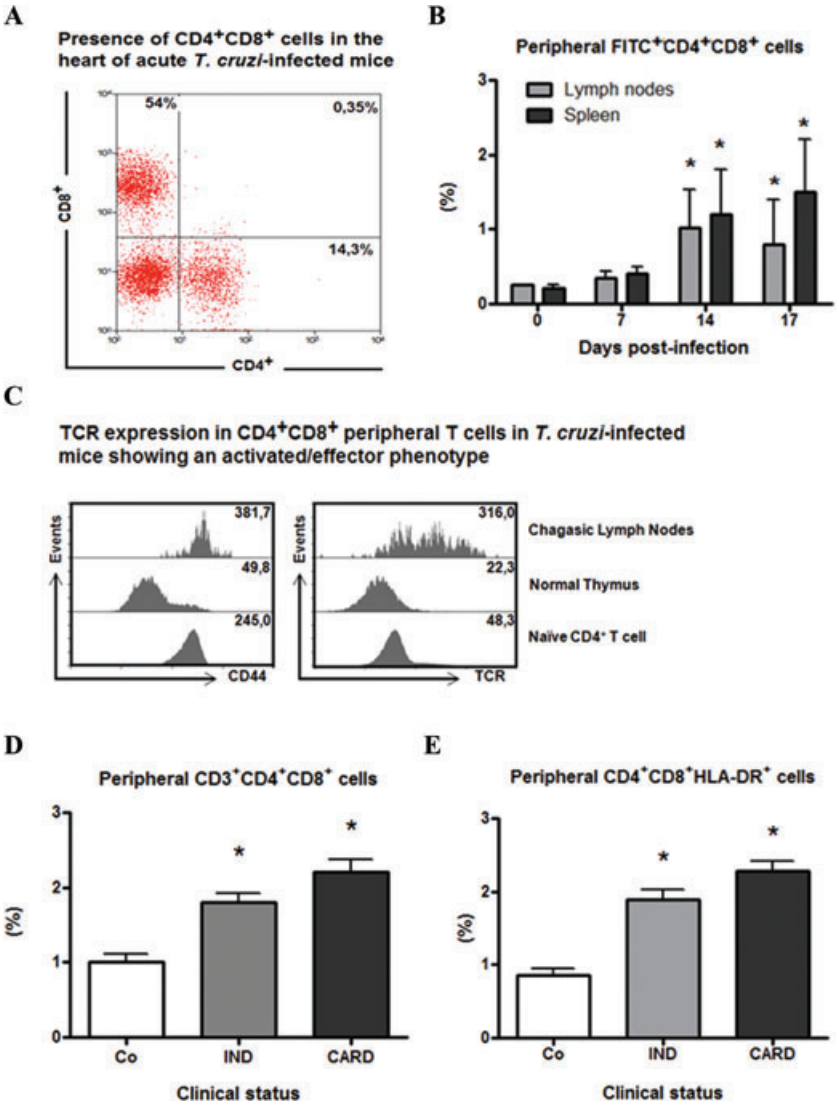

Figure 1. Intrathymic and extrathymic $CD4^+CD8^+$ T cells during experimental and human *T. cruzi* infection. (A) Representative dot plot showing the presence of $CD4^+CD8^+$ cells in the heart during acute *T. cruzi* infection. $CD4^+CD8^+$ cells were not detectable in control mice. (B) The proportions of immature thymus-derived $CD4^+CD8^+$ lymphocytes progressively increase with the course of infection in both lymph nodes and spleen in *T. cruzi*–infected mice. Control and *T. cruzi* acutely infected mice received intrathymic injection of FITC, and 16 hours later were analyzed by flow cytometry to detect $CD4^+CD8^+FITC^+$ as recent thymic emigrant cells in peripheral lymphoid organs. (C) DP cells show an activated/effector phenotype in the periphery of *T. cruzi*–infected mice with high levels of TCR expression. Lymphocytes were isolated from the thymus, subcutaneous lymph nodes, and spleen, during acute phase. Representative histograms of CD44 (left panel); TCR expression levels in $CD4^+CD8^+$ T cells from chagasic lymph nodes and normal thymus, and naive $CD4^+$ T cells from noninfected mice as a control (right panel). The values in the upper-right corner indicate the mean fluorescence intensity from the expression of the markers in each histogram. Differences between chagasic DP from peripheral lymph nodes *versus* normal thymic DP cells and naive T cells from the spleen are significant ($P < 0.05$). (D) Proportion of peripheral blood $CD4^+CD8^+$ cell subset within $CD3^+$ T lymphocytes from healthy human individuals (Co), asymptomatic/indeterminate (IND), or with myocardiopathy (CARD) chronic chagasic patients. Cells were analyzed by flow cytometry ($n = 13$–15 individuals per group). $^*P < 0.05$ versus Co individuals. (E) Proportion of peripheral blood $CD4^+CD8^+$ HLA-DR^+ cell subset on $CD3^+$ T lymphocytes from healthy individuals (Co), asymptomatic/indeterminate (IND), or with myocardiopathy (CARD) chronic chagasic patients. Cells were analyzed by flow cytometry ($n = 13$–15 individuals per group). $^*P < 0.05$ versus Co individuals.

activation pathway of these cells during *T. cruzi* infection. With an extended period of antigenic stimulation, peripheral DP lymphocytes could promptly reach the threshold of activation required to gain effector/memory functions or, alternatively, to further differentiate into antigen-specific SP cells, with a possible role in cell-mediated immunoprotection.[3] In fact, peripheral DP cells from infected mice have been shown to produce high levels of IFN-γ mRNA.[27] Furthermore, our results indicate that DP cells purified from peripheral lymphoid tissues of infected animals show a cytotoxic capacity comparable to that of naive SP T cells.[27]

As the experimental models indicate a premature release of immature DP thymocytes in both acute and chronic experimental Chagas disease,[25] it was plausible to address whether this phenomenon also occurs in chagasic patients. To evaluate this, we examined the frequency of peripheral blood DP cells in both chronic chagasic patients at the indeterminate phase of disease and in individuals with chronic myocarditis. The results showed a higher percentage of DP cells in cardiac chagasic patients compared with healthy individuals (Fig. 1D). Of most relevance, we found that patients with the cardiac form of Chagas disease presented with higher percentages of peripheral blood HLA-DR$^+$ (MHC class II) DP cells, compared with noninfected individuals (Fig. 1E). More recently, similar results were reported by an independent research group.[28] Of note, Giraldo *et al.* showed that human DP T cells can also recognize a parasite-derived MHC class I epitope during chronic infection, probably contributing to *T. cruzi*–induced cardiopathy.[28]

In addition, we phenotyped extrathymic DP T cells for the expression of VLA-4, an integrin-type receptor of fibronectin that also can be seen as a T cell activation marker.[27] Our findings indicate that the increased percentages of circulating DP cells exhibit a fully activated HLA-DR^{high}/VLA-4^{high} pattern, which is enhanced in patients with the severe cardiac form of chronic Chagas disease.[27] Blood DP cells obtained from infected hosts showed marked functional plasticity, exerting both cytolytic activity and/or Th1 helper activity.[27]

As mentioned previously, *T. cruzi* acute infection results in severe thymic atrophy and an early release of DP T cells with autoreactive TCRs into the periphery.[24] We recently showed that despite the thymic atrophy, the machinery necessary to achieve negative selection remains functional during the acute phase of infection,[27] suggesting that DP cells are shunted, escaping the checkpoints required for maturation of T cells and that normally eliminate T cells that express "forbidden" TCRs. Regardless of the specificity of DP T cells, the expression of both CD4 and CD8 in extrathymic DP cells may diminish the functional threshold for antigen-MHC–specific T cell recognition, and/or decrease the requirements of costimulatory signals provided by antigen-presenting cells (APCs) to generate T cell activation. This activation could induce cytotoxic activity, not only against parasite antigens but also toward cells bearing self-antigens, leading to the formation of cross-reactive or neoepitopes, and thus favoring autoimmune reactions in the target tissues.

Further studies are required to elucidate the mechanisms that allow the emergence of potentially autoreactive DP cells in Chagas disease. Nevertheless, we cannot rule out that SP cells in the periphery can become DP cells after a prolonged antigenic stimulation, which could occur as a result of the persistence of the parasite.We might speculate that SP cells become self-reactive after expression of the other coreceptor, thereby inducing an increase in the affinity of the TCR to self-antigens. However, whatever their origin, the data indicate that activated extrathymic DP T cells might be associated with the development of the cardiac clinical form of the disease.

Of note, several other infectious and autoimmune diseases are characterized by the presence of circulating DP T cells, as summarized in Table 1. Interestingly, the detection of DP T cells in the target organs of some autoimmune diseases, such as thyroiditis or rheumatoid arthritis, suggests a connection with the pathophysiology of the corresponding disease.[29,30] Moreover, extrathymic DP T lymphocytes have been detected in other parasitic diseases, including infections by *Trypanosoma evansi* or *Plasmodium berghei*,[31,32] suggesting that these atypical T cells may represent a compensatory response of the immune system to cope with the pathogens. The fact that extrathymic DP cells during *T. cruzi* or viral infections present with an activated phenotype, and thus might exert cytotoxic activity, supports this notion.[27,28,33,34] Alternatively, it is conceivable that activated DP cells may play a

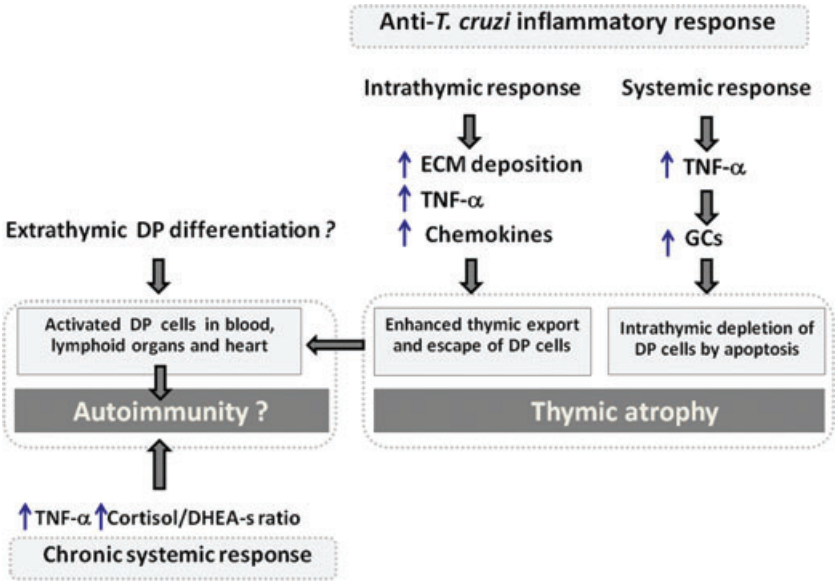

Figure 2. Hypothetical model for the impact of *T. cruzi* infection on DP T cells and their possible role in the immunopathology of Chagas disease. DP $CD4^+CD8^+$ thymocyte subset is severely affected by a dysregulated immunoendocrine circuit of proinflammatory cytokines (mainly TNF-α) and glucocorticoid hormones (GCs). Thymic atrophy is caused by apoptosis of DP thymocytes induced by GCs, although TNF-α together with extracellular matrix (ECM), such as fibronectin and laminin, combined with chemokines, may contribute to enhanced exit of cells from the organ. The abnormal thymic release of DP lymphocytes, in addition to a possible DP differentiation *de novo* in the periphery, may be related with the autoimmune component of Chagas disease. In a chronic scenario, enhanced and sustained levels of TNF-α and enhanced cortisol/DHEA-s ratio may favor the activated status of DP cells and the inflammatory events related with the autoimmune component of disease. The scheme shown in the figure was based on results obtained from studies carried out in C57BL/6 mice infected with the Tulahuen strain and also from human studies.

relevant role in modulating the skewing of adaptive immune responses via cytokine secretion. Accordingly, the cytokines secreted by these cells may address the function of APCs during the early adaptive immune responses, thus providing a link between innate and adaptive immunity. Accordingly, IFN-γ production by DP cells may be involved in the induction of protective/autoreactive Th1 cells.

Table 2. Spearman correlation analysis between percentage of double-positive (DP) $CD4^+CD8^+$ T cells and systemic cytokine and glucocorticoid hormone levels in healthy controls and chagasic patients classified as indeterminate (IND) and with cardiac involvement (CARD)

	Overall ($n = 39$)		Controls ($n = 10$)		IND patients ($n = 10$)		CARD patients ($n = 19$)	
Pair correlation	*P* value	r_s	*P* value	r_s	*P* value	r_s	*P* value	r_s
DPs (%) vs. TNF-α (pg/mL)	**0.0159***	0.40	0.1328	−0.54	0.8123	−0.09	**0.0374***	0.48
DPs (%) vs. IFN-γ (pg/mL)	0.1273	0.25	0.4630	−0.27	0.6821	0.14	0.5835	0.13
DPs (%) vs. Cortisol	0.6012	0.08	0.6436	−0.17	0.8916	0.05	0.8576	−0.04
DPs (%) vs. DHEA-s	**0.0408***	−0.34	**0.0083***	0.82	0.6073	−0.18	**0.0282***	−0.51
DPs (%) vs. Cortisol/DHEA-s	**0.0196***	0.38	**0.0045***	−0.85	0.4483	0.26	**0.0286***	0.51

*P values with statistical significance. Statistical significance was set up at $P < 0.05$.
NOTE: The nonparametric Spearman correlation test was performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, CA www.graphpad.com.

As activated DP cells may hypothetically recognize both class I and class II MHC, they could eliminate activated APCs, favoring parasite persistence during infection.[27]

As discussed previously, although studies in experimental models revealed that during acute infection by *T. cruzi* DP cells come from the thymus, one question that remains elusive is whether DP cells in chronic chagasic patients are thymus-derived or peripherally differentiated, or both. Future studies analyzing T cell receptor exclusion circles in circulating T cells, together with CD45RA/CD45RO markers, should help in further understanding this issue.

DP cells and the immunoendocrine imbalance in human Chagas disease

We have previously shown that a systemic inflammatory milieu, with enhanced levels of TNF-α and IFN-γ, is evident in chagasic patients with severe myocarditis, compared with to healthy subjects.[35] This scenario was paralleled by a disrupted activation of the HPA axis, characterized by decreased concentrations of dehydroepiandrosterone-sulphate (DHEAS) and an unbalanced cortisol/DHEAS ratio, reinforcing the view that severe human Chagas disease is devoid of an adequate anti-inflammatory environment, thus favoring pathology.[35] In keeping with this notion, we observed that extrathymic DP T cells positively correlated with circulating levels of TNF-α and with the cortisol/DHEAS ratio in an overall study population, and within those chagasic patients having cardiopathy (Table 2). By contrast, a negative correlation between extrathymic DP T cells and DHEAS was found in the overall population and also in cardiac patients (Table 2). This raises the question of 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. Chronic stimulation of proinflammatory cytokines may influence the activate state of DP T cells, and at the same time, the cortisol/DHEAS ratio imbalance acts as a permissive scenario to myocarditis development. Further investigation of the relationship among TNF-α, DHEAS, and the cortisol/DHEAS ratio with the phenotype and effector functions of extrathymic DP cells, may help to elucidate the contribution of these cells to the etiology of chagasic carditis.

Conclusions

Overall, the findings support the notion that thymic alterations and the resulting accumulation of extrathymic DP cells during chagasic infection should not be simplistically viewed as a bystander phenomenon but as a relevant pathophysiological component in the course of the disease. Moreover, preserved thymus homeostasis during infections may be relevant for the development of an effective immune response. In this regard, the loss of DP thymocytes during *T. cruzi* infection, as a consequence of immunoendocrine imbalance causing cell death and abnormal exit and peripheral increase of activated DP cells, may also have an impact on tissue damage, thus contributing to the immunopathological events seen during chronic infection. Indeed, an altered cortisol/DHEAS ratio may act as a permissive hormonal environment for the maintenance of the activated state observed in DP cells from chronic chagasic patients. A hypothetical model for the possible role of DP T cells in the immunopathology of Chagas disease is shown in Figure 2. These issues represent an open field for further investigations of Chagas disease.

Acknowledgments

This work was partially funded with grants from CONICET (PIP CONICET 0789) and SeCyT-UNR (MED 244 and MED 245) from Argentina, as well as from CNPq, Faperj, and Oswaldo Cruz Foundation from Brazil, and Amsud/Pasteur network.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Parel, Y. & C. Chizzolini. 2004. CD4+CD8+ double positive (DP) T cells in health and disease. *Autoimmun. Rev.* **3:** 215–220.
2. Antica, M. & R. Scollay. 1999. Development of T lymphocytes at extrathymic sites. *J. Immunol.* **163:** 206–211.
3. Zuckermann, F.A. 1999. Extrathymic CD4/CD8 double positive T cells. *Vet. Immunol. Immunopathol.* **72:** 55–66.
4. Savino, W. & M. Dardenne. 2000. Neuroendocrine control of thymus physiology. *Endocr. Rev.* **21:** 412–443.
5. Savino, W., E. Arzt & M. Dardenne. 1999. Immunoneuroendocrine connectivity: the paradigm of the thymus-hypothalamus/pituitary axis. *Neuroimmunomodulation* **6:** 126–136.
6. Jondal, M., A. Pazirandeh & S. Okret. 2004. Different roles for glucocorticoids in thymocyte homeostasis? *Trends Immunol.* **25:** 595–600.

7. Ashwell, J.D., F.W. Lu & M.S. Vacchio. 2000. Glucocorticoids in T cell development and function. *Annu. Rev. Immunol.* **18:** 309–345.
8. Baseta, J.G. & O. Stutman. 2000. TNF regulates thymocyte production by apoptosis and proliferation of the triple negative ($CD3^-CD4^-CD8^-$) subset. *J. Immunol.* **165:** 5621–5630.
9. Guevara Patiño, J.A., M.W. Marino, V.N. Ivanov & J. Nikolich-Zugich. 2000. Sex steroids induce apoptosis of $CD8^+CD4^+$ double-positive thymocytes via TNF-alpha. *Eur. J. Immunol.* **30:** 2586–2592.
10. Scollay, R. & D.I. Godfrey. 1995. Thymic emigration: conveyor belts or lucky dips? *Immunol. Today.* **16:** 268–273.
11. Berzins, S.P., A.P. Uldrich, J.S. Sutherland, *et al.* 2002. Thymic regeneration: teaching old immune system new tricks. *Trends Mol. Med.* **8:** 469–476.
12. World Health Organization (WHO), Pan American Health Organization (PAHO) Regional Office. 2007. The burden of neglected diseases in Latin America and the Caribbean compared with some other communicable diseases. URL http://www.paho.org/English/AD/DPC/CD/parasit.htm.
13. Coura, J.R. & P.A. Viñas. 2010. Chagas disease: a new worldwide challenge. *Nature* **465:** S6–S7.
14. Cunha-Neto, E., A.M. Bilate, K.V. Hyland, *et al.* 2006. Induction of cardiac autoimmunity in Chagas heart disease: a case for molecular mimicry. *Autoimmunity* **459:** 41–54.
15. Tarleton, R.L. 2001. Parasite persistence in the aetiology of Chagas disease. *Int. J. Parasitol.* **31:** 550–554.
16. Higuchi, M.L. 1997. Chronic chagasic cardiopathy: the product of a turbulent host-parasite relationship. *Rev. Inst. Med.* Trop. **39:** 53–60.
17. Zhang, L. & R.L. Tarleton. 1996. Characterization of cytokine production in murine *Trypanosoma cruzi* infection by in situ immunocytochemistry: lack of association between susceptibility and type 2 cytokine production. *Eur. J. Immunol.* **26:** 102–109.
18. Tarleton, R.L., J. Sun, L. Zhang & M. Postan. 1994. Depletion of T-cell subpopulations results in exacerbation of myocarditis and parasitism in experimental Chagas' disease. *Infect. Immun.* **62:** 1820–1829.
19. Marin-Neto, J.A., E. Cunha-Neto, B.C. Maciel & M.V. Simões. 2007. Pathogenesis of chronic Chagas heart disease. *Circulation* **115:** 1109–1123.
20. Corrêa-de-Santana, E., M. Paez-Pereda, M. Theodoropoulou, *et al.* 2006. Hypothalamus-pituitary-adrenal axis during *Trypanosoma cruzi* acute infection in mice. *J. Neuroimmunol.* **173:** 12–22.
21. Pérez, A.R., E. Roggero, A. Nicora, *et al.* 2007. Thymus atrophy during *Trypanosoma cruzi* infection is caused by an immuno-endocrine imbalance. *Brain Behav. Immun.* **21:** 890–900.
22. Roggero, E., A.R. Pérez, M. Tamae-Kakazu, *et al.* 2006. Endogenous glucocorticoids cause thymus atrophy but are protective during acute *Trypanosoma cruzi* infection. *J. Endocrinol.* **190:** 495–503.
23. Savino, W., D.M. Villa-Verde, D.A. Mendes-da-Cruz, *et al.* 2007. Cytokines and cell adhesion receptors in the regulation of immunity to *Trypanosoma cruzi*. *Cytokine Growth Factor Rev.* **18:** 107–124.
24. Cotta-de-Almeida, V., A. Bonomo, D.A. Mendes-da-Cruz, *et al.* 2003. *Trypanosoma cruzi* infection modulates intrathymic contents of extracellular matrix ligands and receptors and alters thymocyte migration. *Eur. J. Immunol.* **33:** 2439–2448.
25. Mendes-da-Cruz, D.A., J. de Meis, V. Cotta-de-Almeida & W. Savino. 2003. Experimental *Trypanosoma cruzi* infection alters the shaping of the central and peripheral T-cell repertoire. *Microbes Infect.* **5:** 825–832.
26. Argibay, P.F., J.M. Di Noia, A. Hidalgo, *et al.* 2002. *Trypanosoma cruzi* surface mucin TcMuc-e2 expressed on higher eukaryotic cells induces human T cell anergy, which is reversible. *Glycobiology* **12:** 25–32.
27. Morrot, A., E. Terra-Granado, A.R. Pérez, *et al.* 2011. Chagasic thymic atrophy does not affect negative selection but results in the export of activated $CD4^+CD8^+$ T cells in severe forms of human disease. *PLoS Negl. Trop. Dis.* **5:** e1268.
28. Giraldo, N.A., N.I. Bolaños, A. Cuella, *et al.* 2011. Increased $CD4^+/CD8^+$ double-positive T cells in chronic Chagasic patients. *PLoS Negl. Trop. Dis.* **5:** e1294.
29. Iwatani, Y., Y. Hidaka, F. Matsuzuka, *et al.* 1993. Intrathyroidal lymphocyte subsets, including unusual $CD4^+CD8^+$ cells and $CD3^{lo}$TCR alpha-beta^{lo-}CD4–CD8- cells, in autoimmune thyroid disease. *Clin. Exp. Immunol.* **93:** 430–436.
30. De Maria, A., M. Malnati, A. Moretta, *et al.* 1987. $CD3^+4^-8^-$ $WT31^-$(T cell receptor gamma$^+$) cells and other unusual phenotypes are frequently detected among spontaneously interleukin 2-responsive T lymphocytes present in the joint fluid in juvenile rheumatoid arthritis. A clonal analysis. *Eur. J. Immunol.* **17:** 1815–1819.
31. Onah, D.N., J. Hopkin & A.G. Luckins. 1998. Induction of $CD4^+CD8^+$ double positive T cells and increase in $CD5^+$ B cells in efferent lymph in sheep infected with *Trypanosoma evansi*. *Parasite Immunol.* **20:** 121–134.
32. Francelin, C., L.C. Paulino, J. Gameiro & L. Verinaud. 2011. Effects of *Plasmodium berghei* on thymus: high levels of apoptosis and premature egress of $CD4^{(+)}CD8^{(+)}$ thymocytes in experimentally infected mice. *Immunobiol.* **216:** 1148–1154.
33. Nascimbeni, M., S. Pol & B. Saunier. 2011. Distinct $CD4^+CD8^+$ double-positive T cells in the blood and liver of patients during chronic hepatitis B and C. *PLoS ONE.* **6:** e20145.
34. Ortolani, C., E. Forti, E. Radin, *et al.* 1993. Cytofluorimetric identification of two populations of double positive ($CD4^+CD8^+$) T lymphocytes in human peripheral blood. *Biochem. Biophys. Res. Commun.* **191:** 601–609.
35. Pérez, A.R., S.D. Silva-Barbosa, L.R. Berbert, *et al.* 2011. Immunoneuroendocrine alterations in patients with progressive forms of chronic Chagas disease. *J. Neuroimmunol.* **235:** 84–90.
36. Nascimbeni, M., E.C. Shin, L. Chiriboga, *et al.* 2004. Peripheral $CD4^{(+)}CD8^{(+)}$ T cells are differentiated effector memory cells with antiviral functions. *Blood* **104:** 478–486.
37. Howe, R., S. Dillon, L. Rogers, *et al.* 2009. Phenotypic and functional characterization of HIV-1-specific $CD4^+CD8^+$ double-positive T cells in early and chronic HIV-1 infection. *J. Acquir. Immune Defic. Syndr.* **50:** 444–456.
38. Hughes, G.J., A. Cochrane, C. Leen, *et al.* 2008. HIV-1-infected $CD8^+CD4^+$ T cells decay in vivo at a similar rate to infected CD4 T cells during HAART. *AIDS* **22:** 57–65.

39. Ribrag, V., D. Salmon, F. Picard, *et al*. 1993. Increase in double-positive $CD4^+CD8^+$ peripheral T-cell subsets in an HIV-infected patient. *AIDS* **7:** 1530.
40. Wang, X., A. Das, A.A. Lackner, *et al*. 2008. Intestinal double-positive $CD4^+CD8^+$ T cells of neonatal rhesus macaques are proliferating, activated memory cells and primary targets for SIVMAC251 infection. *Blood* **112:** 4981–4990.
41. Furukawa, S., K. Sasai, J. Matsubara, *et al*. 1992. Increase in T cells expressing the gamma/delta receptor and $CD4^+CD8^+$ double-positive T cells in primary immunodeficiency complicated by human T-cell lymphotropic virus type I infection. *Blood* **80:** 3253–3255.
42. Macchi, B., G. Graziani, J. Zhang & A. Mastino. 1993. Emergence of double-positive CD4/CD8 cells from adult peripheral blood mononuclear cells infected with human T cell leukemia virus type I (HTLV-I). *Cell Immunol.* **149:** 376–389.
43. Ciminale, V., M. Hatziyanni, B.K. Felber, *et al*. 2000. Unusual $CD4^+CD8^+$ phenotype in a greek patient diagnosed with adult T-cell leukemia positive for human T-cell leukemia virus type I (HTLV-I). *Leuk. Res*. **24:** 353–358.
44. Ohata, J., M. Matsuoka, T. Yamashita, *et al*. 1999. CD4/CD8 double-positive adult T cell leukemia with preceding cytomegaloviral gastroenterocolitis. *Int. J. Hematol*. **69:** 92–95.
45. Penttilä, J.M., R. Pyhälä, M. Sarvas & N. Rautonen. 1998. Expansion of a novel pulmonary $CD3^{(-)}CD4^{(+)}CD8^{(+)}$ cell population in mice during *Chlamydia pneumoniae* infection. *Infect. Immun.* **66:** 3290–3294.
46. Ghia, P., G. Prato, S. Stella, *et al*. 2007. Age-dependent accumulation of monoclonal $CD4^+CD8^+$ double positive T lymphocytes in the peripheral blood of the elderly. *Br. J. Haematol.* **139:** 780–790.
47. Schlesinger, I., R. Rabinowitz, T. Brenne, *et al*. 1992. Changes in lymphocyte subsets in myasthenia gravis: correlation with level of antibodies to acetylcholine receptor and age of patient. *Neurol*. **42:** 2153–2157.
48. Ferraccioli, G.F., E. Tonutti, L. Casatta, *et al*. 1996. CD4 cytopenia and occasional expansion of $CD4^+CD8^+$lymphocytes in Sjögren's syndrome. *Clin. Exp. Rheumatol.* **14:** 125–130.
49. Ziaber, J., D. Stopczy, H. Tchórzewski, *et al*. 2000. Increased percentage of "double phenotype" form T lymphocytes in blood of patients with multiple sclerosis. *Neurol. Neurochir. Pol.* **34:** 1137–1143.
50. Parel, Y., M. Aurrand-Lions, A. Scheja, *et al*. 2007. Presence of $CD4^+CD8^+$ double-positive T cells with very high interleukin-4 production potential in lesional skin of patients with systemic sclerosis. *Arthritis Rheum.* **56:** 3459–3467.
51. Fozza, C., S. Contini, P. Virdis, *et al*. 2012. Patients with myelodysplastic syndromes show reduced frequencies of $CD4^{(+)}CD8^{(+)}$ double-positive T cells. *Eur. J. Haematol.* **88:** 89–90.
52. Raza, S., S. Naik, V.P. Kancharla, *et al*. 2010. Dual-positive ($CD4^+/CD8^+$) acute adult T-cell leukemia/lymphoma associated with complex karyotype and refractory hypercalcemia: case report and literature. *Case Rep. Oncol.* **3:** 489–494.
53. Kamihira, S., H. Sohda, S. Atogami, *et al*. 1993. Unusual morphological features of adult T-cell leukemia cells with aberrant immunophenotype. *Leuk. Lymphoma* **12:** 123–130.
54. Desfrançois, J., L. Derré, M. Corvaisier, *et al*. 2009. Increased frequency of nonconventional double positive CD4CD8 alpha-beta T cells in human breast pleural effusions. *Int. J. Cancer* **125:** 374–380.
55. Rahemtullah, A., N.L. Harris, M.E. Dorn, *et al*. 2008. Beyond the lymphocyte predominant cell: $CD4^+CD8^+$ T-cells in nodular lymphocyte predominant Hodgkin lymphoma. *Leuk. Lymphoma* **49:** 1870–1878.
56. Rahemtullah, A., K.K. Reichard, F.I. Preffer, *et al*. 2006. A double-positive $CD4^+CD8^+$ T-cell population is commonly found in nodular lymphocyte predominant Hodgkin lymphoma. *Am. J. Clin. Pathol.* **126:** 805–814.