Relationship between Virus Infections and the number of Extrathymic Activated $CD4^+CD8^+$ T Cells

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1 Introduction

Extrathymic DP T cells are increased in some pathophysiological conditions, including infectious diseases by intracellular pathogens, in organs subject to autoimmune attack and in malignant tumors (Morrot *et al*., 2011, 2012; de Meis *et al*., 2012). Recently, there is much evidence that the number of peripheral DP T cells behaving as effector/memory T cells specific for antigens expressed by various viruses increases during virus infections. It is not clear what roles they play, nor has their distribution in the body been well defined (Weiss *et al*., 1998; Howe *et al*., 2009; Nascimbeni *et al*., 2011; Chauhan *et al*., 2012). Where they have been found in animals they have been thought of as memory cells that may participate in adaptive immune responses or alternatively may contribute to the immunopathological responses. Most interestingly, using a *Trypanosoma cruzi* infectious model, we have recently demonstrated that increased percentages of peripheral blood subset of DP T cells exhibiting an activated HLA-DR^+ phenotype are associated with severe cardiac forms of human chronic Chagas disease (Morrot *et al*., 2011; 2012). In what follows we will review current knowledge of the extrathymic DP T cells in humans and animal models.

2 Immune System Resistance against Infection

The primary roles of the immune system are to resist infection and rid the body of cancerous cells. These challenging roles are fulfilled by cooperation between two systems, the innate and the adaptive immune systems. The innate arm provides immediate non-specific protection that is brought into play when pattern recognition receptors on the surface of host cells interact with well-conserved structural components of an infecting pathogen (Chaplin, 2010). The adaptive arm responds to specific pathogen antigens and employs immunological memory to generate long term immunity (Germain *et al*., 2012). It comprises two interdependent types of cells: B-lymphocytes that produce circulating antibodies and T lymphocytes many of which interact directly with target cells (Brink *et al*., 2007; LeBien *et al*., 2008; Pereira *et al*., 2010).

Both of these cell types arise from pluripotent hematopoietic stem cells resident in the bone marrow. T cell maturation depends on the specialized environment of the thymus to achieve lineage commitment and differentiation into mature T cells, whereas B cells generally develop when still in the bone marrow (Lai *et al*., 2008). The thymus is located in the thoracic cavity, and consists of an outer cortex and an inner medulla. It continuously generates T cells, which are then released to the periphery. T-cell development within the thymus is a complex process involving interactions (cross-talk) between different cell types during which interacting stromal cells of various kinds provide the signals required for the correct maturation, expansion and selection of nascent T cell precursors (van Ewijk, 2011). These stromal cells together provide the thymic microenvironment needed for T cell development (Petrie *et al*., 2007).

Defects in thymus function can develop in a number of clinical situations. Such defects in the microenvironment of the thymus can interfere with the development of the adaptive immune system and as a consequence they can lead to life-threatening immunodeficiencies and autoimmune reactions, and interfere with immunological surveillance (Fletcher *et al*., 2011) - the concept that the immune system continually recognizes and removes malignant cells as they arise.

3 Intrathymic T Cell Development

Although the thymus is the primary site of T cell development, it does not contain self-renewing hematopoietic precursor cells, and progenitor cells are constantly recruited from the blood. These cells are characterized by the triple negative phenotype $CD3^-CD4^-CD8^-$ and enter the vascularized microenvironment of the thymus at the junction between the cortex and the medulla. Once they have entered the thymus, they are referred to as early T lineage progenitors (Petrie *et al*., 2007; van Ewijk, 2011). Engagement of their Notch1 receptors with the Delta-like 4 ligands on cortical thymic epithelial cells leads to proliferation of the cells and their commitment to the T cell lineage (Visan *et al*., 2006). They then pursue an ordered sequence of developmental steps (Figure 1), eventually expressing both CD4 and CD8 and complete antigen-specific T cell receptors (Love & Bandhoola, 2011). At this point these cells are referred to as double positive (DP) thymocytes; thereafter they then undergo two successive processes of selection aimed at retaining only those whose randomly assigned TCR specificities are desirable (Love & Bandhoola, 2011). Not all TCRs on DP thymocytes generated by TCR gene rearrangement will still bind to the host's (self) MHC molecules. Cells which have successfully rearranged αβ TCR will die in the thymus cortex if they do not bind self MHC within 3-4 days. Therefore an initial selection process in the thymus will only retain those T cells that bear a αβ TCR that is able to still recognise self MHC. This process is also known as positive selection (van Ewijk, 2011). Positive selection occurs when double positive T cells bind cortical epithelial cells expressing Class I or Class II MHC plus self peptides with a high enough affinity to get the survival signal (Hedrick, 2012).

Those cells with TCRs that fail to match one or other self-peptide/MHC complex adequately fail to receive the necessary survival signals and die within a few days of "death by neglect". The surviving thymocytes are then exposed in the thymus medulla to the second, negative, selection process, which leads to programmed cell death of thymocytes whose TCR have an above-threshold affinity for self-peptide/MHC complexes. This step gets rid of thymocytes that could elicit autoimmune responses. Thus, thymic selection aims to release only T cells that will recognize non-self antigens in the context of self MHC molecules into the periphery (Takahama, 2012). Thymocytes that express a TCR restricted to MHC class I molecules adopt a $CD4^-CD8^+$ phenotype while those with an MHC class II-restricted TCR specificity become $CD4^+CD8^-$ cells (Visan *et al*., 2006; Fiorini *et al*., 2008). After a period of maturation, these naïve single positive cells (i.e. $CD4^-CD8^+$ and $CD4^+CD8^-$ thymocytes) leave the thymic medulla for the periphery in a process that depends on signaling via sphingosine 1-phosphate receptor type 1 (Maeda *et al*., 2010).

Thus, thymocyte development depends on signals provided by a number of different types of stromal cells, notably thymic epithelial cells (TEC), that influence their entry, multiplication, maturation and survival (Ritter *et al*., 1999). The TEC are arranged in a three-dimensional network in which they contact one another via dendrite-like processes, and distinct TEC elements occupy the thymic cortex and medulla, respectively. The mechanisms that control TEC multiplication and maintenance have themselves been the subjects of extensive research, especially as the health of these cells can be affected by the aging process and cancer treatments (Anderson *et al*., 2012).

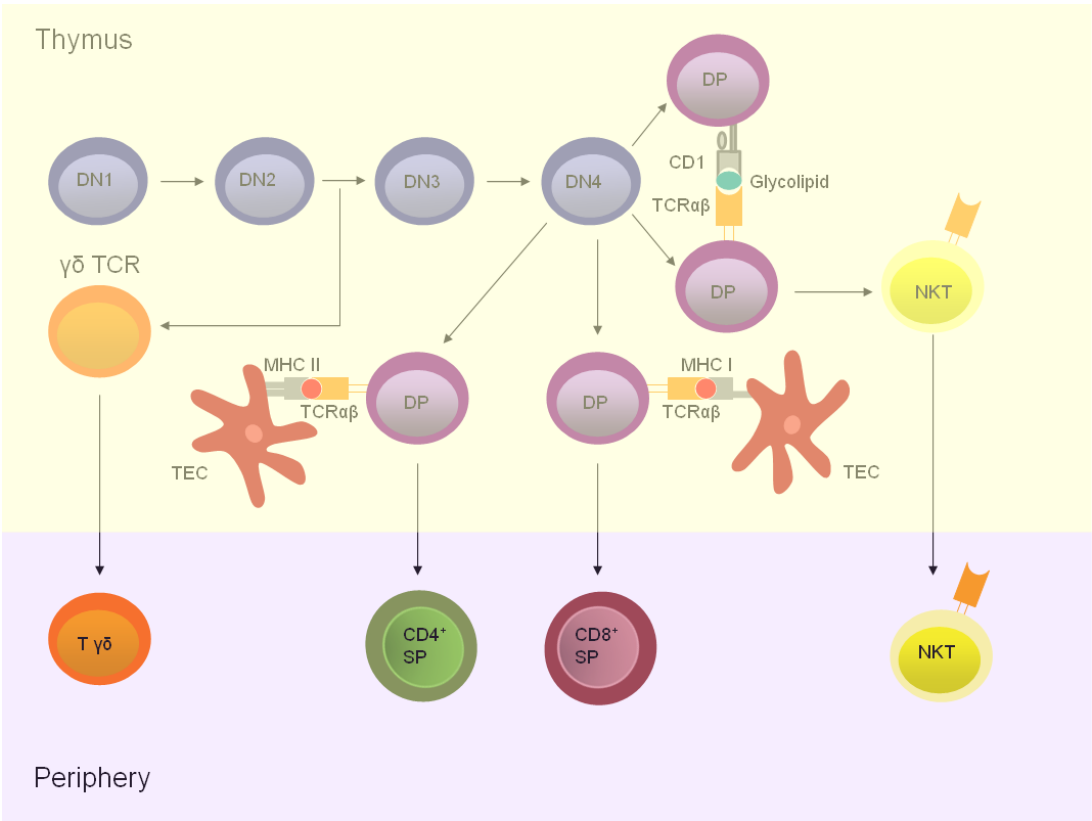

Figure 1: Schematic representation of T cell development in the thymus. A common progenitor of T cells arise in thymus from bone marrow. At the double-negative (DN) stage, gene rearrangement at the TCRβ (T cell receptor), γ and δ gene loci starts. It is believed that strong TCR signaling favors γδ T cell lineage development, while weak TCR signals lead to αβ commitment (Witherden & Havran, 2011). T cells bearing TCR with low avidity to self-peptide–MHC molecules class I or II presented on thymic epithelial cells (TECs) are submitted to the positive selection, becoming $CD8^+$ or $CD4^+$ single-positive (SP) T cells, respectively (Hugo *et al*., 1982; Jameson & Bevan, 1998). Some DP thymocytes express CD1a which binds self glycolipids. Other DP thymocytes able to interact with these cells enter in the NKT lineage (Godfrey & Berzins, 2007). Most NKT that leave thymus complete their maturation process in the periphery. The mature cells can either emigrate from thymus or still remain for a period in the organ (Godfrey & Berzins, 2007).

3.1 Acute Infection by Intracelular Pathogens Can Promote Thymic Atrophy

Atrophy of the thymus has been observed in response to a number of pathogens: viruses (HIV, HCV, rabies virus), parasites (*Trypanosoma cruzi*, *Plasmodium berghei*, *Schistosoma mansoni,* and *Trichinella spiralis*), and fungi (*Paracoccidioides brasiliensis* and *Histoplasma capsulatum*). The basis of this thymic atrophy is not completely clear and may vary (Morrot *et al*., 2012). Nevertheless, there are common histological features, including reduced numbers of cortical thymocytes and loss of the clear-cut distinction between cortex and medulla. In some cases, such as in experimental infection with *Histoplasma*

capsulatum and *Toxoplasma gondii,* the atrophy may be transient (Verinaud *et al*., 1998; Morrot *et al*., 2012; de Meis *et al*., 2012).

Infection-induced thymic atrophy may involve a number of not mutually exclusive factors: reduced entry of precursor cells, a reduced thymocyte capacity for proliferation, increased thymocyte death, and a higher rate of exit of thymocytes from the thymus. The mitogenic responses of thymocytes from mice acutely infected with *T. cruzi* was found to be reduced due to decreased interleukin-2 (IL-2) production, which in turn was associated with elevated levels of IL-10 and interferon-γ (IFN-γ) (de Moraes *et al*., 1994). It seems also that *T. spiralis* infection can alter the proportions of different thymocyte subsets and this is reflected in a reduced capacity of thymocytes to respond to the T-cell mitogen, concanavalin A. In contrast, thymocytes from *S. mansoni*-infected mice apparently exhibited an unaltered proliferative response to concanavalin A (Greene *et al*., 1976). Taken together these findings suggest that some, but not all, parasites cause a reduction in the proliferative ability of thymocytes, which in turn accounts for the resulting thymic atrophy.

In most infectious diseases causing thymic atrophy, the major biological event associated with thymocyte loss is cell death by apoptosis. This is seen for example, in experimental models of *Trypanosoma cruzi* (Henriques-Pons *et al*., 2004; Roggero *et al*., 2009) and *Plasmodium berghei* infection (Carvalho *et al*., 2006; Gameiro *et al*., 2010). Although the main cells that die are $CD4^+CD8^+$ thymocytes, other subsets such as double-negative (DN) T cells and SP cells are also depleted (Morrot *et al*., 2012).

The most likely agents responsible for atrophy and thymocyte death during parasitic infections are glucocorticoid hormones. Serum glucocorticoid levels rise in acute infections and activate caspase-8 and caspase-9 of thymocytes (de Meis *et al*., 2012). Increases in serum glucocorticoids have been observed in experimentally induced malaria, American tripanosomiases or Chagas disease, African trypanosomiases or sleeping sickness, toxoplasmosis, leishmaniasis, and schistosomiasis. In acute experimental *T. cruzi* infections, thymic atrophy and thymocyte depletion are associated with increases in both tumor necrosis factor (TNF-α) and glucocorticoid serum levels (Morrot *et al*., 2012; de Meis *et al*., 2012).

Nevertheless, other mechanisms seem also to be involved, at least in *T. cruzi* infections. Candidates for causing the enhanced thymocyte death include the *T. cruzi*-derived trans-sialidase (Pereira *et al*., 2001; Rosenberg *et al*., 2010), as well as host-derived galectin-3 (Rabinovich *et al*., 2002; Savino *et al*., 2004), extracellular ATP, and androgens. However typical cytotoxic molecules such as Fas and perforin are not involved in thymus atrophy in *T. cruzi* (Henriques-Pons *et al*., 2004).

3.2 Effects of Viral Infection on Extrathymic $CD4^+CD8^+$ T Cells

Although most T cells are either $CD4^+$ or $CD8^+$, a few peripheral $CD4^+CD8^+$ T cells have been found in both humans and animals and higher numbers of such cells are seen in patients with autoimmune diseases and in some acute and chronic viral infections (Weiss *et al*., 1998; Howe *et al*., 2009; Nascimbeni *et al*., 2011; Chauhan *et al*., 2012). Unlike immature thymic DP T cells, peripheral T cells have the properties of mature T cells including antigen-dependent cytokine production and cytolytic activity, and they express markers involved in immunological memory. In pigs $CD4^+CD8^+$ T cells (Th/memory) possess memory, and T-helper and cytolytic properties, and they secrete IFN-γ (Ober *et al*., 1998; De Bruin *et al*., 2000). This T cell subset was associated with protection in pigs vaccinated against pseudorabies virus (Ober *et al*., 1998; De Bruin *et al*., 2000). In intranasally vaccinated and virulent porcine reproductive and respiratory syndrome virus, an arterivirus challenged pigs there is an enhanced frequency of $CD4^+CD8^+$ T cells (Dwivedi *et al*., 2011). Such cells appear to be the progeny of normal antigen-

stimulated single-positive T cells. Human DP T cells can be easily produced from SP T cells *in vitro*, and adoptive transfer experiments in animals indicate that at least some DP cells can arise from $CD4^+$ SP precursors *in vivo* (Parel *et al*., 2004).

Extrathymic DP T cells express variable levels of CD4 and CD8. DP T cells are present in intestinal tissue, particularly in the lamina propria of the jejunum; these cells contain alpha/alpha CD8 homodimers rather than the usual alpha/beta heterodimers found expressed by the perypheral DP T cells (Ortolani *et al*., 1993; Pahar *et al*., 2006). In human immunodeficiency virus infection / acquired immunodeficiency syndrome (HIV/AIDS), the expression of CD4 in DP T cells has a bearing on HIV-1 disease because such cells should be infectable with HIV-1. Indeed, studies indicate that DP T cells, isolated from HIV-1-infected patients, have been shown to contain HIV-1 provirus (Kitchen *et al*., 1998; Hughes *et al*., 2008). Moreover, intestinal DP T cells are at least as susceptible to SIV/HIV-1-mediated depletion as conventional CD4 T cells; similarly, numbers of peripheral blood DP T cells are lower in SIV-infected macaques than in uninfected animals (Mattapallil *et al*., 2000; Veazey *et al*., 2000). Interestingly, examination of a number of HIV-1 subjects receiving a therapeutic vaccine showed that the DP T cells were polyfunctional in that they produced substantial levels of cytokines and also had cytotoxic T lymphocyte (CTL) activity, and this behavior has also been observed in other antigen systems. Importantly, the polyfunctionality of the DP T cells in HIV-1-infected patients was correlated with lower virus loads and nonprogressive disease. However, the antigen-specific properties of DP T cells have not been studied in large numbers of unvaccinated HIV-1-infected individuals (Nam *et al*., 2000; Suni *et al*., 2001; Howe *et al*., 2009).

Most studies of HIV-1 pathology have concentrated on chronically infected patients mainly because few patients are identified soon after infection. Examination of macaque models and naturally infected humans has revealed a dramatic depletion of CD4 T cells in the gut mucosa early in infection (Hirsch *et al*., 2004; Howe *et al*., 2009). It is well established that the virus set point emerging soon after HIV-1 infection predicts later disease progression; hence study of the immune mechanisms in early infected patients, including the T cell polyfunctionality, should reveal important features related to virus set point and thus to disease progression (Mellors *et al*., 1996; Gautam *et al*., 2009).

3.3 The Peripheral $CD4^+CD8^+$ T Cells are Differentiated Effector Memory Cells with Antiviral Functions

During HCV infections, the intra-hepatic and circulating DP T cells are populations of activated, central and effector memory cells, with heterogeneous differentiation patterns, in agreement with findings for the livers of chimpanzees in the acute phase of HCV infection (Nascimbeni *et al*., 2004; 2011). Differences in the measured proportions of circulating DP T cells could depend on differences in methodology and/or patient populations. Using comparable criteria, it has been found that chronically HCV-infected patients contained a variable proportion of DPT cells, and these displayed different patterns of differentiation from those in chronically HBV-infected patients. Since, the differentiation patterns of the DP T cells in HCV and HBV infections are not the same, suggesting the existence of viral specificities (Nascimbeni *et al*., 2011).

Experimentally, HCV infection altered the *ex vivo* phenotype of intra-hepatic DP T cell: the presence of HCV modified the patterns of infiltrating T and DPT cells in the liver, and this effect was not observed in isolated hepatic immune cells and therefore probably resulted from infection to the hepatocytes. Since the changes took place within five days, the peculiar pattern of DPT cells observed in those

patients cannot simply be explained by chronic infection. Instead, the pattern of intra-hepatic DP T cells may result, inter alia, from direct effects of HCV on other local/resident immune cells, or the causes may be more indirect (Nascimbeni *et al*., 2011).

Unlike naïve DP T cells that escape from the thymus, the circulating DP T cells in HCV patients have an activated, differentiated memory phenotype. There is convincing evidence that circulating $CD4^+CD8^+$ T cells are mature memory cells that mount rapid Th1 recall responses to vaccines and viral antigens from self-limited, past, or strongly multiplying, persistent, infections. Moreover, it should be noted that these extra-thymic DP T cells have lower TREC (TCR excision circle) contents and shorter telomeres than SP T cells, indicating at the molecular level that $CD4^+CD8^+$ T cells have experienced more cell divisions than their SP counterparts (Nascimbeni *et al*., 2004). It has also been shown that double-positive $CD4^+CD8^+$ T cells really exist in extrathymic sites in vivo, as these cells were detected in situ at sites of inflammation and viral replication, and could be cloned from chimpanzee liver biopsies during HCV infections. These findings therefore suggest that $CD4^+CD8^+$ T cells contribute early to the immune response to virus infections. There is no doubt that the presence of mature, functional $CD4^+CD8^+$ T cells in peripheral blood and tissues in the infectious diseases challenges our view of the T-cell populations involved in adaptive immune responses (Nascimbeni *et al*., 2011).

Studies on HIV infection model have shown evidences that DP T cells have a memory phenotype and produce cytokines in response to viral antigens. Also it appears that DP T cells can be infected with HIV-1both in vitro and in vivo (Weiss *et al*., 1998). The most significant findings of this study were that HIV-1-reactive cells could be identified within the two previously-defined subpopulations of DP T cells based on their levels of CD4 and CD8 expression, these sharing properties of both conventional CD4 and CD8 SP T cells and therefore could be considered polyfunctional. These HIV-1-specific IFN-γ-producing DP T cells were present at higher frequencies in chronically infected patients than in early in infection, and a greater fraction expressed LAMP markers (Frahm *et al*., 2012; Weiss *et al*., 1998; Chauhan *et al*., 2006).

It is possible that the increased generation of DP T cells as the disease progresses is generally offset by the HIV-1-mediated loss of DP T cells. Detailed phenotypic and functional analysis of HIV-1-specific DP T cells, however, did show up differences between early and chronic infection; these may reflect different mechanisms of induction of these cells and the effects of cumulative antigen dose as the disease progresses. The finding that DP T cells have a unique functional profile argues that independent assessment of DP T cells will be important in further studies of HIV-1 pathogenesis (Ribrag *et al*., 1993).

DPT cells are released from the thymus along with other lymphocytes, and their number non-specifically varies with upon infection, inflammation and other conditions (Table 1). These cells could be produced outside the thymus and/or result from re-expression of CD8 or CD4 from single-positive $CD4^+$ or $CD8^+$ T cells, respectively. Alternatively, it is possible that the production of DPT cells is specifically determined by the microenvironment (including thymic epithelial cells, L-Ti-like cells, local macrophages and DCs) in the thymus, which can itself directly be affected by pathogens, or result from the consequences of infections and other physiological disturbances able to induce thymic involution. The properties of extra-thymic DP T cells indicate a close developmental relationship with conventional CD8 and CD4 T cells.

Pathogens and factors	Changes in DPT populations	References
HCV	Increased percentage of CD4highCD8low in blood and liver from chronically infected patients	Nascimbini et al., 2011
HBV	Similar prcentages of DPT cells in blood from chronically infected patients compared to healthy donors	Nascimbini et al., 2011
Hepatitis virus A59	Depletion of DP thymocytes	Godfraind et al., 1995
Mouse hepatitis virus type 3 (MHV3)	Decrease of DP T cells in liver and thymus	Lamontagne et al.,1997
HIV	Increase of circulating DP T cells	Ribrag et al., 1993; Weiss et al.,1998; Zloza et al., 2003; Howe et al., 2009; Chauhan et al., 2012; Frahm et al., 2012
SIV	Decay rates of infected DP T cells after HAART	Hughes et al., 2008
	Decreased numbers of intestinal DP T cells	Veazey et al., 2000
	Depletion of DP thymocytes	Sodora et al., 2002
SIVmav	DPT cells transiently increased followed by a decrease after 1 month post-infection	Akari et al., 1999
Measle virus	Depletion of DP thymocytes	Auwaerter et al., 1986; Takeuchi et al., 2005; Okamoto et al., 2012
Rabies virus	Depletion of DP thymocytes	Lafon et al., 1994; Markovistz et al., 1994; Cardenas-Palomo et al.,1995
Marburg virus	Increase of circulating DPT cells in cynomolgus macaques.	Fritz et al., 2008
Francisella tularensis	Depletion of DP thymocytes	Chen et al., 2005
Listeria monocytogenes	Depletion of DP thymocytes	Watson et al., 1984
Histoplasma capsulatum	Depletion of DP thymocytes	Watson et al., 1983
Plasmodium berghei	Similar numbers of DPT cells in spleen from infected mice compared to non infected mice	Gameiro et al., 2010
	Presence of DPT cells in mesenteric lymph nodes from infected mice at 14 days post-infection	Francelin et al., 2011
Trypanosoma cruzi	Depletion of DP thymocytes	Savino et al., 1989; Leite de Moraes et al., 1991; Leite de Moraes et al., 1992; Mucci et al., 2002; Savino et al., 2006; de Meis et al., 2012
Trypanosoma cruzi	Increase of circulating DP T cells in mice and humans	Mendes da Cruz et al., 2003; Morrot et al., 2011; Giraldo et al., 2011
Trypanosoma cruzi	Increased frequency of DP T cells in secondary lymph nodes from infected mice	Mendes da Cruz et al., 2003; Cotta de Almeida et al., 2003; Pérez et al., 2012
Glucocorticoids hormones	Depletion of DP thymocytes	Iwata et al., 1996; Ivanov & Nikolić-Zugić, 1998; Zilberman et al., 2004; Corrêa-de-Santana et al., 2006 (a); Corrêa-de-Santana et al., 2006 (b); Wang et al., 2006; Bianchini et al., 2006; Pérez et al., 2007; Roggero et al., 2009; Talabèr et al., 2009; Lepletier et al., 2012; Cohen et al., 2012
ATP	Depletion of DP thymocytes	Mantuano-Barradas et al., 2003; Nagy et al., 2000
Androgens	Depletion of DP thymocytes	Mucci et al., 2005
Trans -sialidase	Depletion of DP thymocytes	Mucci et al., 2002
Galectin-3	Depletion of DP thymocytes	Stillman et al., 2006; Silva-Monteiro et al., 2007
Aging	Increase of peripheral DP T cells	Akari et al., 1997; Nam et al.,1998; Lee et al., 2003 (a); Lee et al., 2003 (b); Ishimoto et al., 2004
Autoimmune diseases	Increase of DP T cells in skin and peripheral blood from patients with atopic dermatitis	Bang et al., 2001
	Increase of DP T cells in peripheral blood from patients with Kawasaki disease	Hirao et al., 1998
	Increased numbers of DP T cells in Myasthenia gravis	Berrih et al., 1981; Fujii et al., 1990; Schlesinger et al., 1992; Truffault et al., 1997
	Presence of DP T cells in thyroid tissues from patients with Graves' and Hashimoto's diseases	Iwatani et al., 1993
	Presence of DP T cells isolated from the joint fluid of patients with Rheumatoid arthritis	De Maria et al., 1987
	Increase of DP T cells in patients with Sjögren's syndrome	Ferraccioli et al., 1996
Neoplasia	Increase of circulating DP T cells	Tamaoki et al., 1997; Borgot et al., 1998; Mizuki et al., 1998; Ohata et al., 1999; Rahemtullah et al., 2006; Rahemtullah et al., 2008; Martinez-Gallo et al., 2008; Desfrançois et al., 2010; Sarrabayrouse et al., 2011; Freeman et al., 2011;

Table 1: Pathogens and factors inducing changes in DP T subsets

It remains to be determined whether the activation of peripheral DP T cells in viral infections depends on the recognition of the cognate peptide/MHC complex (pMHC) expressed by antigen presenting cells (APC). MHC proteins are encoded by a large complex of genes called the major histocompatibility complex (MHC). There are two main structurally and functionally distinct classes of MHC proteins: class I MHC proteins, which present foreign peptides to cytotoxic T cells, and class II MHC proteins, which present foreign peptides to CD4 T helper cells (Barber & Parham, 1993). Importantly, bystander activation, i.e., activation of T cells specific for an antigen X during an immune response against antigen Y

may occur during viral infections. This antigen-independent stimulation of T cells has been best described for CD8 T cells. In the $CD8^+$ compartment, the release of IFN-inducers leads to the production of IL-15, which mediates the proliferation of CD8 T cells, notably memory-phenotype CD8 T cells (Sprent *et al*., 2000). The CD4 T cells also undergo bystander activation by an independent pathway in which common γ-chain cytokines including IL-2 might be involved in bystander activation of $CD4^+$ T cells (Boyman, 2010).

On this line of hypothesis, it is possible that DP T cells arise as a result of in vivo bystander T-cell activation, via cytokines produced during the infection. In this scenario, the activation of these cells which express non-selected αβTCR would circumvents the requirement for specific T-cell receptor stimulation. However, the low frequency of activated DP T cells has rendered it difficult to define the mechanisms and possible in vivo relevance of this nonspecific activation which may be part of certain cellular immune response against specific pathogens. Thus, this identified dual CD4/CD8 expression on activated peripheral T cells have not been reported yet, as they may represent the only known potential links between specific infection signals and the development of specific DP T cell populations derived from thymus.

3.4 Peripheral $CD4^+CD8^+$ T Cells as a Clinical Marker of Disease Progression can Be Correlated with Pathology in Infectious Disease

Besides viral infections, where our knowledge of the extrathymic DP T cells is still limited, it has been show in both murine and human models that infection with the intracelullar protozoan *Trypanosoma cruzi* parasite leads to thymic atrophy and premature release of undifferentiated DP T cells to periphery (Leite-de-Moraes *et al*., 1991; Cotta-de-Almeida *et al*., 2003). It is likely that these events are linked to a particular pathogen-host relationship established during infection. In the *T. cruzi* model, it has been shown that the inflammatory syndrome induced by TNF-α in the acute phase of infection activates the hypothalamus-pituitary-adrenal (HPA) axis with a consequent release of corticosterone. This, in turn, is probably connected to the profound changes in both the lymphoid and non-lymphoid compartments of the thymus, as well as to the outcome of the disease (Roggero *et al*., 2006; Pérez *et al*., 2007; Roggero *et al*., 2009). Nevertheless, other host-derived molecules are involved in the *T. cruzi*-induced thymocyte depletion. This is the case for galectin-3: thymic atrophy is not seen in *T. cruzi*-infected galectin-3 knockout mice (Rabinovich *et al*., 2002; de Meis *et al*., 2012). In addition, the parasite *trans*-sialidase is involved in intrathymic T cell death (Mucci *et al.,* 2002). Moreover, the dramatic changes in the thymic microenvironment occuring after acute infection are also associated with enhanced expression of extracellular matrix ligands and receptors, which correlates with the fibronectin-driven migration of DP thymocytes out of the thymus, and the abnormal increase of immature DP T cells in lymph nodes (Cotta-de-Almeida *et al*., 2003).

It is not clear whether the changes of the thymic microenvironment seen following *T. cruzi* infection also affect the process of intrathymic negative selection of the T cell repertoire. It is generally agreed that clonal deletion normally occurs late in thymocyte development at the DP to SP transition, and takes place at the cortico-medullary junction of the thymic lobules (Sprent *et al*., 2002). In this regard, it has been shown that the thymic metallophilic macrophages located specifically at the cortico-medullary junction are involved in thymocyte maturation (Milicevic *et al*., 2004). There are a number of features that differentiate them from both cortical macrophages and medullary interdigitating cells. In particular, they possess well-developed specialized endocytic compartments for the processing and presentation of anti-

gens by MHC class II molecules (Milicevic *et al*., 2000). It has been shown that infection changes the topological distribution of these metallophilic macrophages. Their numbers increase and although some remain in the cortico-medullary region many more are distributed throughout the cortex. This redistribution of these cells in the thymic compartments during the acute phase of infection may indicate a functional shift of clonal deletion from the cortico-medullary junction to the cortex (Morrot *et al*., 2011).

It is generally accepted that interactions between TEC and thymocytes control the thymic microenvironment and T cell development. Previous studies have shown that the disruption of the normal thymic architecture affects the pattern of expression of autoantigens by TEC, and the functioning of the thymus (Hollander *et al*., 1995; Zuklys *et al*., 2000). Thymic medullary atrophy and decreased expression of Aire and TRAs have been reported in mouse models deficient in a number of genes of the NFκB pathway, such as TRAF6, NIK, RelB or p52, suggesting an important role of this pathway in the development of the thymic medulla (Peterson *et al*., 2008; Zhu *et al*., 2006). We have shown that expression of Aire and of highly selective tissue restricted antigens was readily detectable by real-time PCR in whole thymuses from infected mice, rather similar to controls. These findings suggest that the expression of peripheral antigens in the infected thymuses is sufficient to modulate the induction of tolerance by negative selection (Morrot *et al*., 2011).

As the acute phase of infections progress, thymic atrophy becomes evident and the number of apoptotic intrathymic DP T cells increases (Leite-de-Moraes *et al*., 1992). Although these events may be consequences of the changes observed in the organ, we found that DP depletion was accompanied by sustained expression of Bim, a pro-apoptotic factor essential for negative selection (Liu *et al*., 2003). We also observed using the OTII TCR transgenic system, that administration of the cognate OVA peptide to acutely infected mice undergoing thymic atrophy induced apoptosis of TCR-stimulated semi-mature thymocytes. Taken together, these data indicate that negative selection operates normally during infection-induced thymic atrophy, since DP cells can be negatively selected. This is in agreement with previous work showing that intrathymic mature single-positive $CD4^+$ or $CD8^+$ T cells do not bear forbidden TCR genes unlike their DP counterparts undergoing intrathymic differentiation (Morrot *et al*., 2011).

Despite the fact that the intrathymic checkpoints required to avoid the maturation of T cells that express a forbidden T cell receptor repertoire are active in the acute phase of murine Chagas disease, DP cells are released early on from the infected thymuses (Morrot *et al*., 2011; 2012). Exit during both acute and chronic *T. cruzi* infections may be favored by upregulation of CD62L (L-selectin), which controls lymphocyte homing to lymph nodes. Since DP T cells accumulate progressively in the peripheral lymphoid organs of animals acutely infected *by T. cruzi*, we have examined whether they exhibit activated properties similar to effector/memory single positive T cells. The existence of this unconventional and rare (<5%) lymphocyte population in the periphery has been explained by premature release of DP cells from the thymus into the periphery where their maturation into functionally competent single-positive cells continues (Mendes-da-Cruz *et al*., 2003). There is, however, considerable evidence of an increased frequency of peripheral $CD4^+CD8^+$ T cells during virus infections and during acute *T. cruzi* infection (de Meis *et al*., 2012; Morrot *et al*., 2012). This increase is also seen in the secondary lymph nodes, as we demonstrated in the experimental model of Chagas disease, in which a DP T cell subset increased up to 16 fold in subcutaneous lymph nodes (Morrot *et al*., 2011; Giraldo *et al*., 2011).

Interestingly, despite the expansion of peripheral DP lymphocytes in the experimental model of Chagas disease, these cells develop an activated phenotype, upregulating the activation markers CD44 and CD69, which are tightly linked to the differentiation status of T cells (Morrot *et al*., 2011). In addi-

tion, highly purified (>98%) DP cell populations obtained by cell sorting from infected mice produced high levels of IFN-γ mRNA (Morrot *et al*., 2011). Furthermore, in agreement with previous studies showing that the extrathymic DP cells in *cynomolgus* monkeys and in chimpanzees with experimental hepatitis C virus infections had high cytotoxic activity and effector memory phenotypes, we found that DP T cells, purified from peripheral lymphoid tissues of chagasic animals, had higher cytotoxic activity than naïve single-positive $CD4^+$ or $CD8^+$ T cells (Nam *et al*., 2000; Nascimbeni *et al*., 2004). Most interestingly, patients with the cardiac form of Chagas disease had elevated numbers of peripheral blood HLA-DR^+ DP T cells (Morrot *et al*., 2011). These findings suggest that this T cell subset is associated with the development of the cardiac form of the disease, probably as activated cells (Morrot *et al*., 2011; Giraldo *et al*., 2011).

Together these studies point to a role of the DP T cell subset in the inflammatory processes caused by parasite-driven immune responses. It is possible that co-expression of CD4 and CD8 molecules on the T cell membrane enhances the affinity of the TCR for its target cell. In this way, the simultaneous triggering of CD4 and CD8 co-receptors via MHC-antigen complexes would reduce the threshold for antigen signal and decrease the need for costimulatory signals for T cell activation (Figure 2). This cascade of events would favor DP T cell activation in the presence of low antigen concentrations and could lead to rapid activation of the peripheral DP T subsets at the onset of infection when parasite-derived antigens are limiting. It is thus plausible that these cells play a role in modulating adaptive immune responses by secreting cytokines. Therefore, the cytokines secreted by DP T cells may drive the function of dendritic cells during adaptive immune responses, in that way providing a link between innate and adaptive immunity. In addition, DP T cells may play a part in regulating host immunity. It is also possible that, since DP T cells theoretically recognize both class I and class II MHC complexes, these cells may eliminate activated antigen presenting cells, which would favor the infecting parasite (Figure 2).

In conclusion, the key intrathymic checkpoints necessary for negative selection are effective during acute chagasic thymic atrophy. Despite this, the peripheral DP cells have an activated phenotype similar to that described for activated and memory SP T cells: high IFN-γ production, $CD44^+CD69^+$ expression and cytotoxic activity (Morrot *et al*., 2011). This is particularly important, as we have shown that the appearance of fully activated peripheral DP T cells is associated with the cardiac form of chronic Chagas disease (Morrot *et al*., 2011; Giraldo *et al*., 2011). The presence of these peripheral, mature and activated DP lymphocytes challenges current ideas about the T cell populations involved in adaptive immune responses during *T. cruzi* infections and suggests that DP T cells participate from early on in the immune response against such infections. Therefore, although the *in vivo* function of this DP T cell population remains to be defined, the fact that they have potentially auto reactive TCR may contribute to the imunopathological events in both murine and human Chagas disease. Observations correlating changes in levels of DP T cell subsets with the extent of myocardial damage in the cardiac disease could permit the identification of a clinical marker of disease progression, and might help in the design of alternative treatments for the chronic morbidity of the disease. These studies should provide fundamental insight into virus-host relationship established during infection.

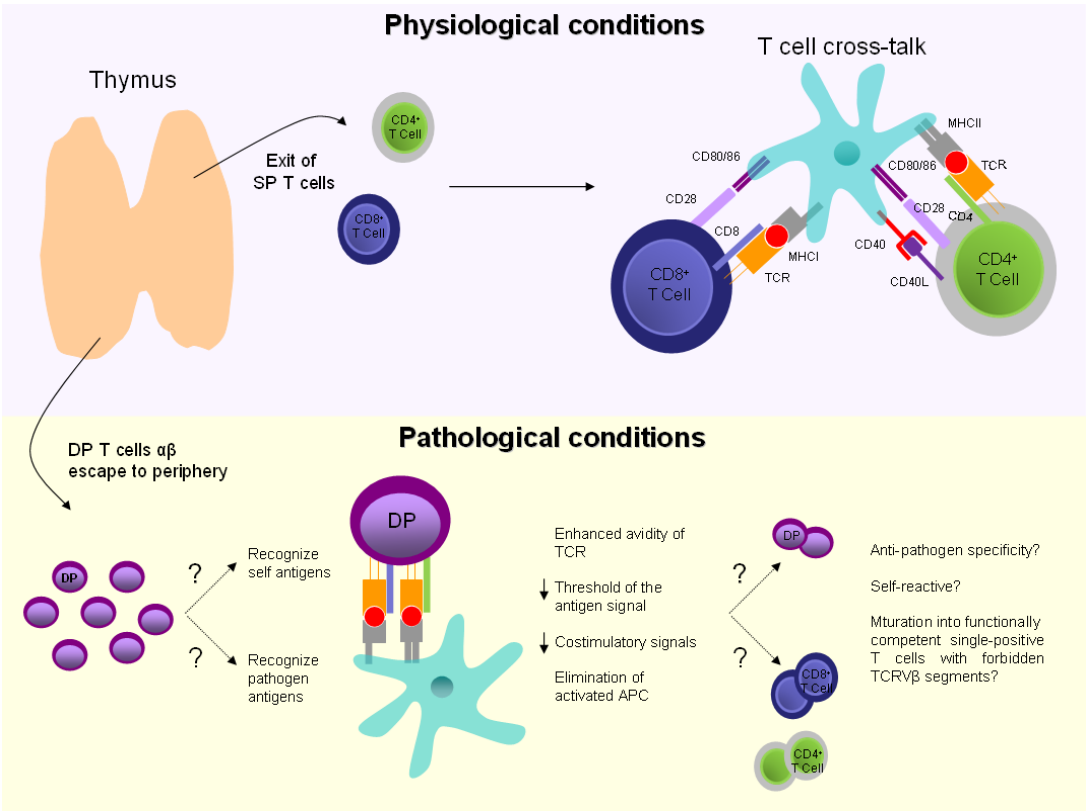

Figure 2: Proposed mechanisms for the role of extrathymic DP T cells. In physiological conditions, activated dendritic cells upregulate MHC and the costimulatory molecules CD80/86 and CD40 (Banchereau *et al*., 2000). Recognition of peptide antigens expressed on MHC class II molecules by antigen-specific $CD4^+$ T cells, together with signals provided by the costimulatory molecules leads to activation of $CD4^+$ T cells and the upregulation of CD40 ligand (CD40L) (Banchereau *et al*., 2000). This activation results in the increased expression of several costimulatory molecules, including CD80/CD86 at DCs, which in turn allows these cells to activate $CD8^+$ T cells (Bennet *et al*., 1998). In a pathological setting it is possible that co-expression of CD4 and CD8 molecules on the T cell membrane prematurely released from thymus would enhance the avidity of the TCR during the priming of double-positive $CD4^+CD8^+$ T cells which in turns could reduce the threshold of the costimulatory signals in the T cell-dendritic cell immunological synapses. Since the DP T cells would be able to recognize both peptide-class I and II MHC complexes at the same time, these cells could quickly eliminate the antigen presenting cells before the activation of single-positive T cells. In this context, the DP T cells would possibly have an immunodominant effect that could play a role in modulating adaptive immune responses. These prematurely released activated DP thymocyte cells could also maturate in the periphery into functionally competent single positive cells able to recognize pathogen antigens. Alternatively, they could express forbidden αβTCR with self-antigen specificities that could be determinant to induce autoimmune responses.

4 Concluding Remarks

The classical paradigm for T-cell dynamics suggests that the resolution of a primary infection is followed by the generation of a long-lived and stable pool of memory lymphocytes. Since the physical basis of the response is stochastic, very limited alteration in this repertoire is expected to occur due to alterations of the dynamics of the lymphoid tissues. Understanding the basis of stochasticity in lymphocyte fluctuations that occur secondary to viral and other pathogen infections will hopefully improve our knowledge on the lymphocyte dynamics, and this will likely be useful for the development of protective immune responses necessary to control persistent infections.

Overall the dynamics of T-cell expansion and contraction in persistent pathogen infections also reflects the redistribution of lymphocyte subsets in the lymphoid tissues along the infection. Fluctuations in the lymphocyte cell population may reflect specific and coordinated responses to the pathogens in the lymphoid organs. In this regard, correlations between the changes in the numbers of DP T cell subsets and the extent of inflammatory pathology may represent a clinical marker of disease progression in viral and other pathogen infections and may help the design of novel therapeutic approaches for controlling infectious diseases.

References

Anderson, G. & Takahama, Y. (2012). Thymic epithelial cells: working class heroes for T cell development and repertoire selection. *Trends in Immunology*, 33(6), 256-263.

Akari, H., Nam, K.H., Mori, K., Otani, I., Shibata, H., Adachi, A., Terao, K. & Yoshikawa, Y. (1999). Effects of SIVmac infection on peripheral blood CD4+CD8+ T lymphocytes in cynomolgus macaques. *Clinical Immunology,* 91(3), 321-329.

Akari, H., Terão, K., Murayama, Y., Nam, K.H. & Yoshikawa, Y. (1997). Peripheral blood CD4+CD8+ lymphocytes in cynomolgus monkeys are of resting memory T lineage. *International Immunology.* 9(4), 591-597.

Auwaerter, P.G., Kaneshima, H., McCune, J.M., Wiegand, G. & Griffin, D.E. (1996). Virus measles infection of the thymic epithelium in the SCID-hu mice lead to thymocyte apoptosis. *Journal of Virology,* 70, 3734–3740.

Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y.J., Pulendran, B., Palucka, K. (2000). Immunobiology of dendritic cells. *Annual Review of Immunology,* 18,767-811.

Bang, K., Lund, M., Wu, K., Mogensen, S.C. & Thestrup-Pedersen K. (2001). CD4+ CD8+ (thymocyte-like) T lymphocytes present in blood and skin from patients with atopic dermatitis suggest immune dysregulation. *British Journal of Dermatology,* 144, 1140 –1147.

Barber, L.D. & Parham, P. (1993). Peptide Binding to Major Histocompatibility Complex Molecules. *Annual Review of Cell Biology,* 9, 163-206.

Bennett, S.R., Carbone, F.R., Karamalis, F., Flavell, R. A., Miller, J.F., Heath, W. R. (1998). Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature,* 393(6684), 478-480.

Berrih, S., Gaud, C., Bach, M.A., Le Brigand, H., Binet, J.P. & Bach, J.F. (1981). Evaluation of T cell subsets in myasthenia gravis using anti-T cell monoclonal antibodies. *Clinical Experimental Immunology*, 4, 1 –8.

Bianchini, R., Nocentini, G., Krausz, L.T., Fettucciari, K., Coaccioli, S., Ronchetti, S. & Riccardi, C. (2006). Modulation of pro- and antiapoptotic molecules in double-positive (CD4+CD8+) thymocytes following dexamethasone treatment. *Journal of Pharmacology and Experimental Therapeutics,* 319(2), 887-897.

Boyman, O. (2010). Bystander activation of CD4+ T cells. *European Journal of Immunology,* 40(4), 936-939.

Brink, R., Phan, T.G., Paus, D. & Chan, T.D. (2007). Visualizing the effects of antigen affinity on T-dependent B-cell differentiation. *Immunology & Cell Biology*, 86(1), 31-39.

Cardenas-Palomo, L.F., de Souza-Matos, D.C., Chaves-Leal, E., Bertho, A.L. & Marcovistz, R. (1995). Lymphocyte subsets and cell proliferation analysis in rabies-infected mice. *Journal of Clinical & Laboratory Immunology,* 46, 49–61.

Carvalho, L.J., Ferreira-da-cruz, M.F., Daniel-Ribeiro, C.T., Pelajo-Machado, M. & Lenzi, H.L. (2006). *Plasmodium berghei* ANKA infection induces thymocyte apoptosis and thymocyte depletion in CBA mice. *Memórias do Instituto Oswaldo Cruz*, 101(5), 523-528.

Chaplin, D.D. (2010). Overview of the immune response. *Journal of Allergy and Clinical Immunology*, 125(2 Suppl 2), S3-23.

Chauhan, N.K., Vajpayee, M., Mojumdar, K., Singh, R. & Singh A. (2012). Study of CD4+CD8+ double positive T-lymphocyte phenotype and function in Indian patients infected with HIV-1. *Journal of Medical Virology*, 84(6), 845-856.

Chen, W., Kuolee, R., Austin, J.W., Shen, H., Che, Y. & Conlan, J.W. (2005). Low dose aerosol infection of mice with virulent type A *Francisella tularensis* induces severe thymus atrophy and CD4+CD8+ thymocyte depletion. *Microbial Pathogenesis,* 39, 189–196.

Cohen, O., Ish-Shalom, E., Kfir-Erenfeld, S., Herr, I., & Yefenof, E. (2012). Nitric oxide and glucocorticoids synergize in inducing apoptosis of CD4+8+ thymocytes: implications for 'Death by Neglect' and T-cell function. *International Immunology,* Sep 4.

Corrêa-de-Santana, E. Pinto-Mariz, F. & Savino, W. (2006). Immunoneuroendocrine interactions in Chagas disease. *Annals of New York Academy of Science,* 1088, 274-283.

Corrêa-de-Santana, M., Paez-Pereda, M., Theodoropoulou, M., Kenji Nihei, O., Gruebler, Y., Bozza, M., Arzt, E., Villa-Verde, D.M., Renner, U., Stalla, J., Stalla, G.K. & Savino, W. (2006). Hypothalamus-pituitary-adrenal axis during *Trypanosoma cruzi* acute infection in mice. *Journal of Neuroimmunology,* 173(1-2), 12-22.

Cotta-de-Almeida, V., Bonomo, A., Mendes-da-Cruz, D.A., Riederer, I., De Meis, J., Lima-Quaresma, K.R., Vieira-de-Abreu, A., Villa-Verde, D.M. & Savino, W. (2003). Trypanosoma cruzi infection modulates intrathymic contents of extracellular matrix ligands and receptors and alters thymocyte migration. *European Journal of Immunol*ogy, 33, 2439–2448.

Dardenne, M. (1991) Studies on the thymus in Chagas' disease. II. Thymocyte subset fluctuations in Trypanosoma cruzi-infected mice: Relationship to stress. *Scandinavian Journal of Immunology,* 33, 267–275.

De Bruin, T. G., E. M. Van Rooij, *et al*. (2000). Cytolytic function for pseudorabies virus-stimulated porcine CD4+ CD8dull+ lymphocytes. *Viral Immunology,* 13(4), 511-520.

De Maria, A., Malnati, M., Moretta, A., Pende, D., Bottino, C., Casorati, G., Cottafava, F., Melioli, G., Mingari, M.C., Migone, N., Romagnani, S. & Moretta, L. (1987). CD3+4-8- WT31-(T cell receptor gamma+) cells and other unusualphenotypes are frequently detected among spontaneously interleukin 2-responsive T lymphocytes present in the joint fluid in juvenile rheumatoid arthritis. A clonal analysis. *European Journal of Immunology,* 17, 1815 –1819.

de Meis, J., Farias-de-Oliveira, A.D., Panzenhagen N.P.H., Maran, N., Villa-Verde, D.M., Morrot, A. & Savino W. (2012). Thymus atrophy and double-positive escape are common features in infectious diseases. *Journal of Parasitology Research*, 574020.

de Moraes, M.D.C.L., Minoprio, P., Dy, M., Dardenne, M., Savino, W. & Hontebeyrie-Joskowicz, M. (1994). Endogenous IL-10 and IFN-γ production controls thymic cell proliferation in mice acutely infected by *Trypanosoma cruzi*. *Scandinavian Journal of Immunology,* 39(1), 51–58.

Desfrançois, J., Moreau-Aubry, A., Vignard, V., Godet, Y., Khammari, A., Dréno, B., Jotereau, F. & Gervois, N. (2010). Double positive CD4CD8 alphabeta T cells: a new tumor-reactive population in human melanomas. *PLoS ONE* 5(1), e8437.

Dwivedi, V., C. Manickam, *et al*. (2011). Cross-protective immunity to porcine reproductive and respiratory syndrome virus by intranasal delivery of a live virus vaccine with a potent adjuvant. *Vaccine,* 29(23), 4058-4066.

Ferraccioli, G.F., Tonutti, E., Casatta, L., Pegoraro, I., De Vita, S., Sala, P., Ravaioli, T. & Bartoli, E. (1996). CD4 cytopenia and occasional expansion of CD4+CD8+lymphocytes in Sjögren's syndrome. *Clinical and Experimenatl Rheumatology,* 14(2), 125-130.

Fiorini, E., Ferrero, I., Merck, E., Favre, S., Pierres, M., Luther, S.A. & MacDonald, H.R. (2008). Cutting edge: thymic crosstalk regulates delta-like 4 expression on cortical epithelial cells. *Journal of Immunology*, 181 (12), 8199-8203.

Fletcher, A.L., Calder, A., Hince, M.N., Boyd, R.L. & Chidgey, A.P. (2011). The contribution of thymic stromal abnormalities to autoimmune disease. *Critical Reviews in Immunology*, 31(3), 171-187.

Frahm, M.A., Picking, R.A., Kuruc, J.D., McGee, K.S., Gay, C.L., Eron, J.J., Hicks, C.B., Tomaras G.D. & Ferrari, G. (2012). CD4+CD8+ T cells represent a significant portion of the anti-HIV T cell response to acute HIV infection. *Journal of Immunology,* 188(9), 4289-4296.

Francelin, C., Paulino, L.C., Gameiro, J. & Verinaud, L. (2011). Effects of Plasmodium berghei on thymus: high levels of apoptosis and premature egress of CD4(+)CD8(+) thymocytes in experimentally infected mice. *Immunobiology,* 216(10), 1148-1154.

Freeman, L.M., Lam, A, Petcu E, Smith R, Salajegheh A, Diamond P, Zannettino A, Evdokiou, A, Luff, J., Wong, P.F., Khalil, D., Waterhouse, N., Vari, F., Rice, A.M., Catley, L., Hart, D.N. & Vuckovic, S. (2011). Myeloma-induced alloreactive T cells arising in myeloma-infiltrated bones include double-positive CD8+CD4+ T cells: evidence from myeloma-bearing mouse model. *Journal of Immunology,* 187(8), 3987-3996.

Fritz, E.A., Geisbert, J.B., Geisbert, T.W., Hensley, L.E. & Reed, D.S. (2008). Cellular immune response to Marburg virus infection in cynomolgus macaques. *Viral Immunology,* 21(3), 355-63.

Fujii, Y., Hayakawa, M., Inada, K. & Nakahara, K. (1990). Lymphocytes in thymoma: association with myasthenia gravis is correlated with increased number of single-positive cells. *European Journal of Immunology,* 20(10), 2355-2358.

Gameiro, J., Nagib, P.R., Andrade, C.F., Villa-Verde, D.M., Silva-Barbosa, S.D., Savino, W, Costa, F.T. & Verinaud L. (2010). Changes in cell migration-related molecules expressed by thymic microenvironment during experimental Plasmodium berghei infection: consequences on thymocyte development. *Immunology*, 129(2), 248-256.

Gao, W., Pereira & M.A. (2001). Trypanosoma cruzi trans-sialidase potentiates T cell activation through antigen-presenting cells: role of IL-6 and Bruton's tyrosine kinase. *European Journal of Immunology*, 31(5), 1503-1512.

Gautam, R., Gaufin, T., Butler, I., Gautam, A., Barnes, M., Mandell, D., Pattison, M., Tatum, C., Macfarland, J., Monjure, C., Marx, P.A., Pandrea, I. & Apetrei, C. (2009). Simian immunodeficiency virus SIVrcm, a unique CCR2-tropic virus, selectively depletes memory CD4+ T cells in pigtailed macaques through expanded coreceptor usage in vivo. *Journal of Virology*, 83(16), 7894-7908.

Germain, R.N., Robey, E.A. & Cahalan, M.D. (2012). A decade of imaging cellular motility and interaction dynamics in the immune system. *Science*, 336(6089), 1676-1681.

Giraldo, N.A., Bolaños, N.I., Cuellar, A., Guzman, F., Uribe, A.M., Bedoya, A., Olaya, N., Cucunubá, Z.M., Roa, N., Rosas, F., Velasco, V., Puerta, C.J. & González, J.M. (2011). Increased CD4+/CD8+ double-positive T cells in chronic Chagasic patients. *PLoS Neglected Tropical Diseases*, 5(8), e1294.

Godfrey, D. I. & Berzins, S. P. (2007). Control points in NKT-cell development. *Nature Reviews Immunology,* 7, 505-518.

Greene, B.M. & Colley, D.G. (1976). Eosinophils and immune mechanisms. III. Production of the lymphokine eosinophil stimulation promoter by mouse T lymphocytes. *Journal of Immunology*, 116(4), 1078-1083.

Hedrick, S.M. (2012). Positive selection in the thymus: an enigma wrapped in a mystery. *Journal of immunology,* 188(5), 2043-2045.

Henriques-Pons, A., De Meis, J., Cotta-De-Almeida, V., Savino, W. & Araújo-Jorge, T.C. (2004). Fas and perforin are not required for thymus atrophy induced by *Trypanosoma cruzi* infection. *Experimental Parasitology*, 107(1-2), 1-4.

Hirao, J. & Sugita, K. (1998). Circulating CD4+CD8+ T lymphocytes in patients with Kawasaki disease. *Clinical Experimental Immunology,* 111, 397–401.

Hirsch, V.M. (2004). What can natural infection of African monkeys with simian immunodeficiency virus tell us about the pathogenesis of AIDS? *AIDS Reviews*, 6(1), 40-53.

Hollander, G.A., Wang, B., Nichogiannopoulou, A., Platenburg, P.P., van Ewijk, W., Burakoff, S.J., Gutierrez-Ramos, J.C. & Terhorst, C. (1995). Developmental control point in induction of thymic cortex regulated by a subpopulation of prothymocytes. *Nature*. 373, 350–353.

Howe, R., Dillon, S., Rogers, L., Palmer, B., MaWhinney, S., Blyveis, N., Schlichtemeier, R., D'Souza, M., Ingoldby, L., Harwood, J.E., Rietmeijer, C., Ray, G., Connick, E., Wilson, C.C. (2009). Phenotypic and functional characterization of HIV-1-specific CD4+CD8+ double-positive T cells in early and chronic HIV-1 infection. *Journal of Acquired Immune Deficiency Syndromes*, 50(5), 444-456.

Hughes, G.J., Cochrane, A., Leen, C., Morris, S., Bell, J.E. & Simmonds, P. (2008). HIV-1-infected CD8+CD4+ T cells decay in vivo at a similar rate to infected CD4 T cells during HAART. *AIDS*, 22(1), 57-65.

Hugo, P., Kappler, J. W., Godfrey, D.I., Marrack, P. C. (1992). A cell line that can induce thymocyte positive selection. *Nature*, 360, 679–682.

Ishimoto, Y., Tomiyama-Miyaji, C., Watanabe, H., Yokoyama, H., Ebe, K., Tsubata, S., Aoyagi, Y. & Abo, T. (2004). Age-dependent variation in the proportion and number of intestinal lymphocyte subsets, especially natural killer T cells, double-positive CD4+ CD8+ cells and B220+ T cells, in mice. *Immunology,* 113(3), 371-377.

Ivanov, V.N. & Nikolić-Zugić, J. (1998). Biochemical and kinetic characterization of the glucocorticoid-induced apoptosis of immature CD4+CD8+ thymocytes. *International Immunology*, 10(12), 1807-1817.

Iwata, M., Ohoka, Y., Kuwata, T. & Asada A. (1996). Regulation of T cell apoptosis via T cell receptors and steroid receptors. *Stem Cells,* 14(6), 632-641.

Iwatani, Y., Hidaka, Y., Matsuzuka, F., Kuma, K. & Amino, N. (1993). Intrathyroidal lymphocyte subsets, including unusual CD4+ CD8+ cells and CD3loTCR alpha beta loy-CD4–CD8- cells, in autoimmune thyroid disease. *Clinical and Experimental Immunology,* 93, 430–436.

Jameson, S. C., Bevan, M. J. (1998). T-cell selection. *Current Opinion in Immunology,* 10, 214–219.

Jacques, B., Francine, B., Christophe, C., Jean, D., Serge, L.,Yong-Jun, L., Bali, P., Karolina, P. (2000). Immunobiology of Dendritic Cells. *Annual Review of Immunology,* 18, 767-811.

Kitchen, S.G., Korin, Y.D., Roth, M.D., Landay, A. & Zack, J.A. (1998). Costimulation of naïve CD8+ lymphocytes induces CD4 expression and allows human immunodeficiency virus type 1 infection. *Journal of Virology*, 72(11), 9054-9060.

Lafon, M., Scott-Algara, D., Marche, P.N., Cazenave, P.A. & Jouvin-Marche, E. (1994). Neonatal deletion and selective expansion of mouse T cells by exposure to rabies virus nucleocapsid superantigen. *Journal of Experimental Medicine,* 180, 1207–1215.

Lai, A.Y. & Kondo, M. (2008). T and B lymphocyte differentiation from hematopoietic stem cell. *Seminars in Immunology,* 20(4), 207-212.

Lamontagne, L., Massicotte, E. & Page, C. (1997). Mouse hepatitis viral infection induces an extrathymic differentiation of the specific intrahepatic alpha beta-TCRintermediate LFA-1high T-cell population. *Immunology,* 90(3), 402-411.

LeBien, T.W. & Tedder, T.F. (2008). B lymphocytes: how they develop and function. *Blood*, 112(5), 1570-1580.

Lee, W.W., Nam, K.H., Terao, K. & Yoshikawa, Y. (2003). Possible role of genetic factor(s) on age-related increase of peripheral CD4+CD8+ double positive T cells in cynomolgus monkeys. *Journal of Experimental Animal Science,* 2(4), 309-316.

Lee, W.W., Nam, K.H., Terao, K., Akari, H. & Yoshikawa Y. (2003). Age-related increase of peripheral CD4+ CD8+ double-positive T lymphocytes in cynomolgus monkeys: longitudinal study in relation to thymic involution. *Immunology,* 109(2), 217-25.

Leite de Moraes, M.C, Hontebeyrie-Joskowicz ,M., Leboulanger, F., Savino, W., Dardenne, M. & Lepault, F (1991). Studies on the thymus in Chagas' disease. II. Thymocyte subset fluctuations in *Trypanosoma cruzi*-infected mice: Relationship to stress. *Scandinavian Journal of Immunology,* 33, 267–275.

Leite-de-Moraes, M.C., Hontebeyrie-Joskowicz, M., Dardenne, M. & Savino W. (1992). Modulation of thymocyte subsets during acute and chronic phases of experimental *Trypanosoma cruzi* infection. *Immunology*, 77, 95–98.

Lepletier, A., de Frias, C.V., Morrot, A. & Savino, W. (2012). Thymic atrophy in acute experimental Chagas disease is associated with an imbalance of stress hormones. *Annals of the New York Academy of Sciences*, 1262(1), 45-50.

Liu, X., Dai, S., Zhu, Y., Marrack, P. & Kappler, J.W. (2003). The structure of a Bcl-xL/Bim fragment complex: implications for Bim function. *Immunity,* 19, 341–352.

Love, E. P. & Bhandoola, A. (2011). Signal integration and crosstalk during thymocyte migration and emigration. *Nature Reviews Immunology*, 11, 469–477.

Macarthur, K.L., Wu, C.H. & Wu, G.Y. (2012). Animal models for the study of hepatitis C virus infection and replication. *World Journal of Gastroenterology*, 18(23), 2909-13.

Maeda, Y., Seki, N., Sato, N., Sugahara, K. & Chiba K. (2010). Sphingosine 1-phosphate receptor type 1 regulates egress of mature T cells from mouse bone marrow. *International Immunology*, 22(6), 515-525.

Mantuano-Barradas, M., Henriques-Pons, A., Araújo-Jorge, T. C., Di Virgilio, F., Coutinho-Silva, R. & Persechini, P.M. (2003). Extracellular ATP induces cell death in CD4+/CD8+ double-positive thymocytes in mice infected with *Trypanosoma cruzi*. *Microbes and Infection,* 5(15), 1363-1371.

Markovistz, R., Bertho, A.L. & Matos, D.C. (1994). Relationship between apoptosis and thymocyte depletion in rabies-infected mice. *Brazilian Journal of Medical and Biological Research*, 27, 1599–1603.

Martinez-Gallo, M., Puy, C., Ruiz-Hernandez, R., Rodriguez-Arias, J.M., Bofill, M., Nomdedeu, J.F., Cigudosa, J.C., Rodriguez-Sanchez, J.L. & de la Calle-Martin, O. (2008). Severe and recurrent episodes of bronchiolitis obliterans organising pneumonia associated with indolent CD4+ CD8+ T-cell leukaemia. *European Respiratory Journal*, 31(6), 1368-1372.

Mattapallil, J.J., Reay, E. & Dandekar, S. (2000). An early expansion of CD8alphabeta T cells, but depletion of resident CD8alphaalpha T cells, occurs in the intestinal epithelium during primary simian immunodeficiency virus infection. *AIDS*, 14(6), 637-646.

Mellors, J.W., Rinaldo, C.R., Gupta, P., White, R.M., Todd, J.A. & Kingsley, L.A. (1996). Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science*, 272(5265), 1167-1170.

Mendes-da-Cruz, D.A., de Meis, J., Cotta-de-Almeida, V. & Savino, W. (2003). Experimental Trypanosoma cruzi infection alters the shaping of the central and peripheral T-cell repertoire. *Microbes and Infection,* 5,825–832.

Milicevic, N.M. & Milicevic, Z. (2000). Ultrastructural identification of specialized endocytic compartments in macrophages of the thymic cortico-medullary zone and germinal centers of peripheral lymphatic organs of the rat. *Annals of Anatomy,* 182, 471–478.

Milicevic, N.M. & Milicevic, Z. Thymus cell-cell interactions. (2004). *International Review of Cytology,* 235, 1–52.

Mizuki, M., Tagawa, S., Machii, T., Shibano, M., Tatsumi, E., Tsubaki, K., Tako. H., Yokohama, A., Satou, S., Nojima, J., Hirota, T. & Kitani, T. (1998). Phenotypical heterogeneity of CD4+CD8+ double-positive chronic T lymphoid leukemia. *Leukemia,* 12(4), 499-504.

Morrot, A., Barreto de Albuquerque, J., Berbert, L.R., de Carvalho Pinto, C.E., de Meis, J. & Savino, W. (2012). Dynamics of Lymphocyte Populations during Trypanosoma cruzi Infection: From Thymocyte Depletion to Differential Cell Expansion/Contraction in Peripheral Lymphoid Organs. *The American Journal* of *Tropical Medicine and Hygiene,* 747185.

Morrot, A., Terra-Granado E., Pérez, A.R., Silva-Barbosa, S.D., Milićević, N.M., Farias-de-Oliveira, D.A., Berbert, L.R., De Meis, J., Takiya, C.M., Beloscar, J., Wang, X., Kont, V., Peterson, P., Bottasso, O. & Savino, W. (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 Neglected Tropical Diseases*, 5(8), e1268.

Mucci, J., Hidalgo, A., Mocetti, E., Argibay, P. F., Leguizamon, M. S. & Campetella, O. (2002). Thymocyte depletion in *Trypanosoma cruzi* infection is mediated by *trans*-sialidase-induced apoptosis on nurse cells complex. *Proceedings of the National Academy of Sciences of the United States of America,* 99, 3896–3901.

Mucci, J., Mocetti, E., Leguizamon, M. S. & Campetella, O. (2005). A sexual dimorphism in intrathymic sialylation survey is revealed by the *trans*-sialidase from *Trypanosoma cruzi. Journal of Immunology,* 174, 4545–4550.

Nagy, P.V., Fehér, T., Morga, S. & Matkó J. (2000). Apoptosis os murine thymocytes induced by extracellular ATP is dose- and cytosolic pH-dependent. *Immunology Letters,* 72(1), 23-30.

Nam, K., Akari, H., Terao, K., Shibata, H., Kawamura, S. & Yoshikawa, Y. (2000). Peripheral blood extrathymic CD4+CD8+ T cells with cytotoxic activity are from the same lineage as CD4(+)CD8(-) T cells in cynomolgus monkeys. *International Immunology,* 12(7), 1095-1103.

Nam, K.H., Akari, H., Terao, K., Ohto, H., Itagaki, S. &Yoshikawa Y. (1998). Age-dependent remodelinh of peripheral CD4+CD8+ T lymphocytes in cynomolgus monkeys. *Developmental & Comparative Immunology,* 22(2), 239-248.

Nascimbeni, M., Pol, S. & Saunier, B. (2011). Distinct CD4+ CD8+ double-positive T cells in the blood and liver of patients during chronic hepatitis B and C. *PLoS ONE,* 6(5), e20145.

Nascimbeni, M., Shin, E.C., Chiriboga, L., Kleiner, D.E. & Rehermann, B. (2004). Peripheral CD4+CD8+ T cells are differentiated effector memory cells with antiviral functions. *Blood*, 104(2), 478-486.

Ober, B. T., Summerfield A., Mattlinger C., Wiesmüller K.H., Jung G., Pfaff E., Saalmüller A.& Rziha H.J. (1998). Vaccine-induced, pseudorabies virus-specific, extrathymic CD4+CD8+ memory T-helper cells in swine. *Journal of Viro*logy, 72(6), 4866-4873.

Ohata, J., Matsuoka, M., Yamashita, T., Tojo, A., Tani, K. & Asano, S. (1999). CD4/CD8 double-positive adult T cell leukemia with preceding cytomegaloviral gastroenterocolitis. *International Journal of Hematology,* 69(2), 92-95.

Okamoto, Y., Vricella, L.A., Moss, W.J. & Griffin, D.E. (2012). Immature CD4(+)CD8(+) Thymocytes Are Preferentially Infected by Measles Virus in Human Thymic Organ Cultures. *PLoS ONE,* 7(9), e45999.

Ortolani, C., Forti, E., Radin, E., Cibin, R. & Cossarizza, A. (1993). Cytofluorimetric identification of two populations of double positive (CD4+CD8+) T lymphocytes in human peripheral blood. *Biochemical and Biophysical Research Communication*, 191(2), 601-609

Pahar, B., Lackner, A.A. & Veazey, R.S. (2006). Intestinal double-positive CD4+CD8+ T cells are highly activated memory cells with an increased capacity to produce cytokines. *European Journal of Immuno*logy, 36(3), 583-592.

Parel, Y. & Chizzolini, C. (2004). CD4+ CD8+ double positive (DP) T cells in health and disease. *Autoimmunity Reviews*, 3(3), 215-220.

Pereira, J.P., Kelly, L.M. & Cyster J.G. (2010). Finding the right niche: B-cell migration in the early phases of T-dependent antibody responses. *International Immunology,* 22(6), 413-419.

Pérez, A.R., Roggero, E., Nicora, A., Palazzi, J., Besedovsky, H.O., Del Rey, A. & Bottasso, O.A. (2007). Thymus atrophy during Trypanosoma cruzi infection is caused by an immuno-endocrine imbalance. *Brain, Behavior and Immunity*, 21(7), 890-900.

Peterson, P., Org, T. & Rebane, A. Transcriptional regulation by AIRE: molecular mechanisms of central tolerance. (2008). *Nature Reviews Immunol*ogy, 8, 948–957.

Petrie, H.T. & Zúñiga-Pflücker, J.C. (2007). Zoned out: functional mapping of stromal signaling microenvironments in the thymus. *Annual Review in Immunology*, 25, 649-679.

Rabinovich, G.A., Baum, L.G., Tinari, N., Paganelli, R., Natoli, C., Liu, F.T. & Iacobelli, S. (2002) Galectins and their ligands: amplifiers, silencers or tuners of the inflammatory response? *Trends in Immunology,* 23(6):313-20.

Rahemtullah, A., Harris, N.L., Dorn, M.E., Preffer, F.I. & Hasserjian, R.P. (2008). Beyond the lymphocyte predominant cell: CD4+CD8+ T-cells in nodular lymphocyte predominant Hodgkin lymphoma. *Leukemia and Lymphoma,* 49(10), 1870-1878.

Rahemtullah, A., Reichard, K.K., Preffer, F.I., Harris, N.L. & Hasserjian, R.P. (2006). A double-positive CD4+CD8+ T-cell population is commonly found in nodular lymphocyte predominant Hodgkin lymphoma. *American Journal of Clinical Pathology,* 126(5), 805-814.

Ribrag, V., Salmon, D., Picard, F., Guesnu, M., Sicard, D. & Dreyfus, F. (1993). Increase in double-positive CD4+CD8+ peripheral T-cell subsets in an HIV-infected patient. *AIDS,* 7(11), 1530.

Ritter, M. A. & Palmer, D.B. (1999). The human thymic microenvironment: New approaches to functional analysis. *Seminars in Immunology,* 11, 13–21.

Roggero, E., Pérez, A.R., Bottasso, O.A., Besedovsky, H.O. & Del Rey, A. (2009). Neuroendocrine-immunology of experimental Chagas' disease. *Annals of the New York Academy of Sciences,* 1153, 264-271.

Roggero, E., Pérez, A.R., Tamae-Kakazu, M., Piazzon, I., Nepomnaschy, .I, Besedovsky, H.O, Bottasso, O.A. & del Rey, A. (2006). Endogenous glucocorticoids cause thymus atrophy but are protective during acute Trypanosoma cruzi infection. *Journal of Endocrinology*, 190(2), 495-503.

Rosenberg, C.S., Martin, D.L. & Tarleton, R.L. (2010). CD8+ T cells specific for immunodominant trans-sialidase epitopes contribute to control of Trypanosoma cruzi infection but are not required for resistance. *Journal of Immunology*, 185(1), 560-568.

Sarrabayrouse, G., Corvaisier, M., Ouisse, L.H., Bossard, C., Le Mével, B., Potiron, L., Meurette, G., Gervois, N. & Jotereau F. (2011). Tumor-reactive CD4+ CD8αβ+ CD103+ αβT cells: a prevalent tumor-reactive T-cell subset in metastatic colorectal cancers. *International Journal of Cancer,* 128(12), 2923-3932.

Savino, W. (2006). The thymus is a common target organ in infectious Diseases. *PLoS Pathogens*, 2(6), p. e62.

Savino, W., Leite de Moraes, M.C., Hontebeyrie-Joskowicz, M. & Dardenne, M. (1989). Studies on the thymus in Chagas' disease. I. Changes in the thymic microenvironment in mice acutely infected with *Trypanosoma cruzi. European Journal of Immunology,* 19, 1727–1733.

Savino,W., Mendes-Da-Cruz, D.A., Smaniotto, S., Silva-Monteiro, E. & Villa-Verde, D.M. (2004). Molecular mechanisms governing thymocyte migration: combined role of chemokines and extracellular matrix. *Journal of Leukocyte Biology*, 75(6), 951-961.

Schlesinger, I., Rabinowitz, R., Brenner, T., Abramsky, O. & Schlesinger M. (1992). Changes in lymphocyte subsets in myasthenia gravis: correlation with level of antibodies to acetylcholine receptor and age of patient. 42(11), 2153-2157.

Silva-Monteiro, E., Reis Lorenzato, L., Kenji Nihei, O., Junqueira, M., Rabinovich, G.A., Hsu, D.K., Liu, F.T., Savino, W., Chammas, R. & Villa-Verde, D.M. (2007). Altered expression of galectin-3 induces cortical thymocyte depletion and premature exit of immature thymocytes during *Trypanosoma cruzi* infection. *Ameriacan Journal of Pathology,* 170(2), 546-556.

Sodora, D.L., Milush, J.M., Ware, F., Wozniakowski, A., Montgomery, L., McClure, H.M., Lackner, A.A., Marthas, M., Hirsch, V., Johnson, R.P., Douek, D.C. & Koup, R.A. (2002). Decreased levels of recent thymic emigrants in peripheral blood of simian immunodeficiency virus-infected macaques correlates with alterations within the thymus. *Journal of Virol*ogy, 76, 9981–9990.

Sprent, J. & Kishimoto, H. (2002). The thymus and negative selection. *Immunological Reviews,* 185, 126–135.

Sprent, J., Zhang, X., Sun, S., Tough, D. (2000). T-cell proliferation in vivo and the role of cytokines. *Philosophical Transactions of the Royal Society of London,* 355(1395), 317-322.

Stillman, B.N., Hsu, D.K., Pang, M., Brewer, C.F., Johnson, P., Liu, F.T. & Baum, L.G. (2006). Galectin-3 and galectin-1 bind distinct cell surface glycoprotein receptors to induce T cell death. *Journal of Immunology,* 176(2), 778-789.

Suni, M.A., Ghanekar, S.A., Houck, D.W., Maecker, H.T., Wormsley, S.B., Picker, L.J., Moss, R.B. & Maino, V.C. (2001). CD4(+)CD8(dim) T lymphocyte exhit enhanced cytokine expression, proliferation and cytotoxic activity in response to HCMV and HIV-1 antigens. *European Journal of Immunology,* 31(8), 2512-2520.

Tamaoki, J., Chiyotani, A., Nagai, A. & Konno, K. (1997). Invasive thymoma with CD4+CD8+ double-positive T cell lymphocytosis. *Respiration,* 64(2), 176-178.

Talabér, G., Boldizsár, F., Bartis, D., Pálinkás, L., Szabó, M., Berta, G., Sétáló, G. Jr., Németh, P. & Berki, T. (2009). Mitochondrial translocation of the glucocorticoid receptor in double-positive thymocytes correlates with their sensitivity to glucocorticoid-induced apoptosis. *International Immunology,* 21(11), 1269-1276.

Takahama, Y. (2012). T lymphocyte development and selection in the thymic microenvironments. *Nature Reviews Immunology,* 84(3), 177-1782.

Takeuchi, K., Takeda, M., Miyajima, N., Ami, Y., Nagata, N., Suzaki, Y., Shahnewaz, J., Kadota, S. & Nagata, K. (2005). Stringent requirement for the C protein of wild-type measles virus for growth both in vitro and in macaques. *Journal of Virology,* 79, 7838–7844.

Taniguchi, R.T. & Anderson, M.S. (2010). The role of Aire in clonal selection. *Immunology and Cell Biology*, 89(1), 40-44.

Truffault, F., Cohen-Kaminsky, S., Khalil, I., Levasseur, P., & Berrih-Aknin, S. (1997). Altered intrathymic T-cell repertoire in human myasthenia gravis. *Annals of Neurology,* 41(6), 731-741.

Van Ewijk, W. (2011). The thymus: "Interactive teaching during lymphopoiesis". *Immunology Letters*, 138(1), 7-8.

Veazey, R.S., Mansfield, K.G., Tham, I.C., Carville, A.C., Shvetz, D.E., Forand, A.E. & Lackner, A.A. (2000). Dynamics of CCR5 expression by CD4+ T cells in lymphoid tissues during simian immunodeficiency virus infection. *Journal of Virology,* 74(23), 11001-11007.

Verinaud, L., Da Cruz-Höfling, M.A., Sakurada, J.K., Rangel, H.A., Vassallo, J., Wakelin, D., Sewell, H.F. & Camargo, I.J. (1998). Immunodepression induced by Trypanosoma cruzi hepatitis virus type 3 is associated with thymus apoptosis. *Clinical and Diagnostic Laboratory Immunology,* 5(2), 186-191.

Visan, I., Yuan, J.S., Tan, J.B., Cretegny, K. & Guidos, C.J. (2006). Regulation of intrathymic T-cell development by Lunatic Fringe- Notch1 interactions. *Immunological Reviews*, 209, 76-94.

Watson, S.R., Miller, T.B., Redington, T. J. & Bullock, W.E. (1983). Immunoregulation in experimental disseminated histoplasmosis: Flow microfluorometry (FMF) studies of the Thy and Lyt phenotypes of T lymphocytes from infected mice. *Journal of Immunol*ogy, 131, 984–990.

Watson, S.R.., Redington, T.J., Miller, T.B. & Bullock, W.E. (1984). Flow microfluorometry analysis of alterations in T-lymphocyte subsets during murine listeriosis. *Infection and Immunity,* 45, 372–377.

Weiss, L., Roux, A., Garcia, S., Demouchy, C., Haeffner-Cavaillon, N., Kazatchkine, M.D., Gougeon, M.L. (1998). Persistent expansion, in a human immunodeficiency virus-infected person, of V beta-restricted CD4+CD8+ T lymphocyte that express cytotoxicity-associated molecules and are commited to produce interferon-gamma and tumor necrosis factor-alpha. *Journal of Infectious Diseases,* 178(4), 1158-1162.

Witherden, D.A. & Havran, W.L. (2011). Molecular Aspects of Epithelial γδ T Cell Regulation. *Trends in Immunology,* 32(6), 265–271.

Zhu, M., Chin, R.K., Christiansen, P.A., Lo, J.C., Liu, X., Ware, C., Siebenlist, U. & Fu, Y.X. (2006). NF-kappaB2 is required for the establishment of central tolerance through an Aire-dependent pathway. *Journal of Clinical Invesigation,* 116, 2964–2971.

Zilberman, Y., Zafrir, E., Ovadia, H., Yefenof, E., Guy, R. & Sionov, R.V. (2004). The glucocorticoid receptor mediates the thymic epithelial cell-induced apoptosis of CD4+8+ thymic lymphoma cells. *Cellular Immunology,* 227(1), 12-23.

Zuklys, S., Balciunaite, G., Agarwa‚l A., Fasler-Kan, E., Palmer, E. & Holländer, G.A. (2000). Normal thymic architecture and negative selection are associated with Aire expression, the gene defective in the autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). *Journal of Immunol*ogy, 165, 1976–1983.