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Review Article

The Development of Unconventional Extrathymic Activated $CD4^+CD8^+$ T Cells in Chagas Disease

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The numbers of extrathymic $CD4^+CD8^+$ double-positive (DP) T cells are augmented in various pathophysiological conditions, such as infectious diseases caused by intracellular pathogens, organs subjected to autoimmune attack and malignant tumors. The roles performed by extrathymic DP T cells are not clear, and it is not known how they are distributed in the body. In animals they have been considered memory cells involved in adaptive immune responses against virus infections or participating in pathological responses. In experimental *Trypanosoma cruzi* infections, there is a severe thymic atrophy and this results in the release of activated DP T cells to peripheral organs. In severe cardiac forms of human chronic Chagas disease activated HLA-DR^+ DP T cells are present in the blood. In investigating the basis of premature thymocyte release during chagasic thymic atrophy we found that the parasite *trans*-sialidase (TS) altered intrathymic thymocyte maturation and was associated with increased numbers of recent T cells in peripheral lymphoid organs. In what follows we propose to describe what is known about the origin of the extrathymic DP T cells in human Chagas disease and animal models of the disease.

1. T Cell Development in the Thymus

The formation of mature lineage-committed T cells requires the specialized environment of the thymus, whereas B cells generally develop while still in the bone marrow [1, 2]. The thymus, located in the thoracic cavity, comprises an outer cortex and an inner medulla. T cells are continuously generated in the thymus and released to the periphery. The complex developmental process responsible for generating T cells within the thymus depends on signals from stromal cells that direct the maturation, expansion, and selection of T cell precursors [3, 4]. The stromal cells thus provide a thymic microenvironment appropriate for T cell development [5, 6]. Thymus function is defective in a variety of clinical situations. Such a defective thymic microenvironment can prevent the development of the adaptive immune system, cause life-threatening immunodeficiencies and autoimmune reactions, and inhibit the process of immunological surveillance [7–12], which is thought to be responsible for recognizing and removing malignant cells as they arise.

Although the thymus does not contain self-renewing hematopoietic precursor cells, progenitor cells are constantly recruited from the blood. The recruited cells have a triple-negative $CD3^-CD4^-CD8^-$ phenotype and penetrate the vascularized microenvironment of the thymus at the boundary between the cortex and medulla. Once in the thymus, they designated early T lineage progenitors [4, 5, 13], and engagement of their Notch1 receptors with the delta-4-like ligands on cortical thymic epithelial cells induces proliferation of the cells and results in their commitment to the T cell lineage [14–17]. After an ordered sequence of developmental steps (Figure 1), they eventually express both the CD4 and CD8 antigens and intact antigen-specific T cell receptors [18, 19]. These cells referred, to as DP thymocytes, are submitted to two successive selection steps that lead to the elimination of cells whose randomly assigned T cell receptor (TCR) specificities are undesirable [18].

Many of the TCRs on DP thymocytes generated by TCR gene rearrangement are unable to bind self major histocompatibility complex (MHC) molecules, and such cells

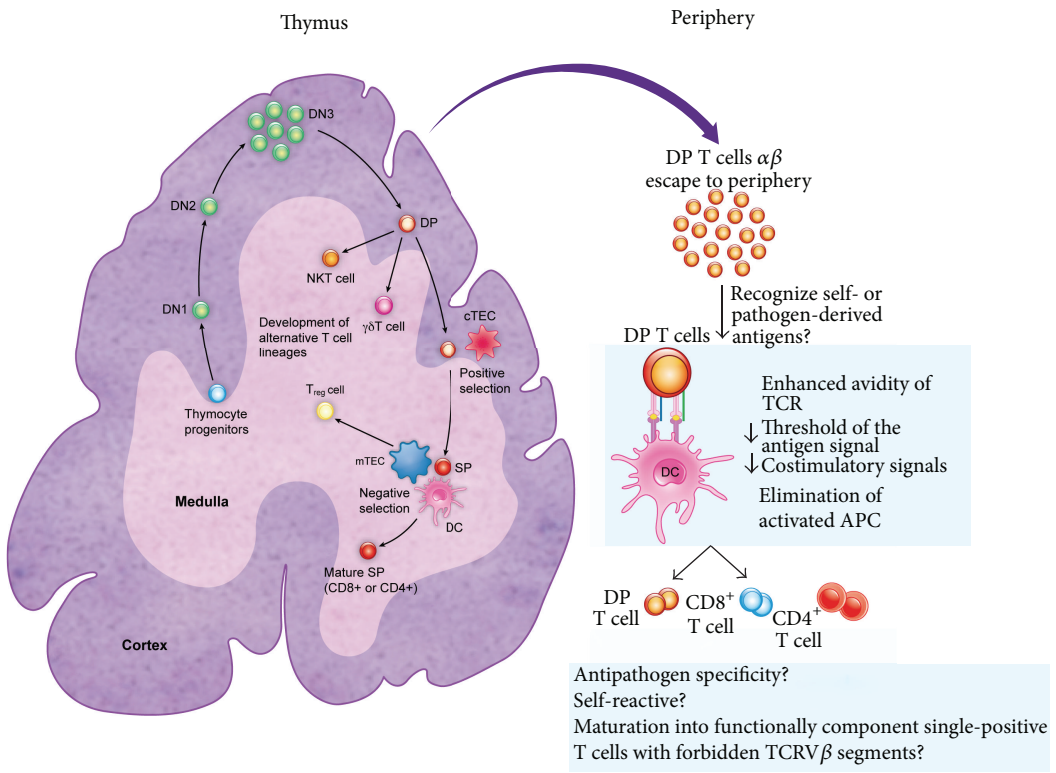

Figure 1: Schema of T cell development in the thymus and of the proposed role of extrathymic DP T cells. Left figure: progenitors of $CD4^{-}CD8^{-}$ (DN) T cells enter the thymus from bone marrow via the large blood vessels at the border between cortex and medulla and move towards the capsule of the organ. Rearrangement at the T cell receptor (TCRβ): γ and δ gene loci commence, and strong TCR signaling favors development of the $\gamma\delta$ T cell lineage, while weak TCR signals lead to $\alpha\beta$ commitment [20]. Only cells that rearrange their T cell receptor β (TCRβ) chain proliferate extensively and give rise to $CD4^{+}CD8^{+}$ double-positive (DP) thymocytes. These cells occupy much of the cortex and move around slowly while their TCRα chains are rearranged. During this phase, weak interaction in the cortex between the newly formed T cell receptors (TCRs) on cortical thymocytes and self-peptides bound to major histocompatibility complex (MHC) proteins on cortical thymic epithelial cells (TECs) leads to survival and maturation of the thymocytes. Thereafter only T cells bearing TCR with a low avidity for any of the self-peptide-MHC class I or II molecules presented on thymic epithelial cells (TECs) are subjected to selection, becoming $CD8^{+}$ or $CD4^{+}$ single-positive (SP) T cells [18]. Other cells with high avidity for any of the self-peptide-MHC class I or II molecules die by apoptosis via negative selection in the central medullary region of the thymus or are alternatively differentiated into regulatory T cells [21, 22]. Some DP thymocytes express CD1a, which binds self-glycolipids, and other DP thymocytes that are able to interact with these cells enter the NKT lineage. Most NKT cells that exit the thymus become mature in the periphery. Mature cells within the thymus can either emigrate from the thymus or remain for a period in that organ [23]. Right figure: in a pathological setting, the severe thymic atrophy leads to release of activated DP T cells to the periphery. In Chagas disease, it has been well demonstrated that the *T. cruzi trans*-sialidase affects the T cell intrathymic development resulting in an increase in the number of $CD4^{+}8^{+}$ double-positive recent thymic emigrants to the secondary lymphoid tissues. In the periphery, the coexpression of CD4 and CD8 molecules on the DP T cells released from the thymus may increase the affinity of the TCR during priming and so may reduce the threshold for costimulatory signals at T cell-dendritic cell immunological synapses [24, 25]. Since the DP T cells express TCR complex with CD4 and CD8 molecules, they would be able to recognize both peptide-class I and II MHC receptors at the same time, and these cells could rapidly eliminate APCs before other single-positive T cells could even be activated [26, 27]. Thus the DP T cells might have an immunodominant effect that could increase adaptive immune responses. The prematurely released activated DP thymocyte cells might also mature into functionally competent SP cells able to recognize pathogen antigens. Alternatively, they could express forbidden $\alpha\beta$TCR with self-antigen specificities and could thus induce autoimmune responses.

will die within 3-4 days. Therefore an initial selection process in the thymus will only retain those T cells that bear a $\alpha\beta$ TCR that is able to still recognise self MHC. This process is known as positive selection [4, 28] and occurs when the T cells bind cortical epithelial cells expressing Class I or Class II MHC plus self-peptides with a sufficiently high affinity to receive a survival signal [29, 30]. The remaining cells die within a few days from "death by neglect" [31, 32]. The surviving thymocytes undergo in the medulla of the thymus a second, negative selection process, which leads programmed cell death of thymocytes whose TCRs have an above-threshold affinity for self-peptide/MHC complexes. In that way thymocytes capable of generating elicit autoimmune responses are removed, and only T cells able to recognize nonself-antigens in the context of self-MHC molecules are released into the periphery [33, 34].

During T cell development, thymocytes that produce a TCR restricted to class I MHC molecules adopt a $CD4^{-}CD8^{+}$

phenotype while those with an MHC class II-restricted TCR specificity turn into $CD4^+CD8^-$ cells [14, 35, 36]. After a time required for maturation, these naïve single-positive (SP) T cells (i.e., $CD4^-CD8^+$ and $CD4^+CD8^-$ thymocytes) exit the thymic medulla and migrate to the periphery in a process that requires signaling via sphingosine 1-phosphate receptor type 1 [37, 38]. In this way, thymocyte development requires signals emanating from a number of different types of stromal cells, particularly thymic epithelial cells (TEC); these influence their entry, division, maturation, and survival [39, 40]. The TECs form a three-dimensional network held together by contacts via dendrite-like processes, and the TEC elements occupying the thymic cortex and medulla are different. The processes involved in TEC multiplication and maintenance have been extensively studied since the functioning of these cells is affected by aging and by anticancer treatments [41–44].

2. Infectious Diseases Can Result in Thymic Atrophy

Several infectious pathogens for example, viruses (HIV, HCV, rabies virus), parasites (*Trypanosoma cruzi*, *Plasmodium berghei*, *Schistosoma mansoni*, and *Trichinella spiralis*), and fungi (*Paracoccidioides brasiliensis* and *Histoplasma capsulatum*) can cause atrophy of the thymus. It is not completely clear how this atrophy occurs and the mechanism may vary [45–53]. However, there are common histological features, such as lower numbers of cortical thymocytes and breakdown of the clear-cut distinction between cortex and medulla. In some cases atrophy may be transient, for example, in experimental infection with *Histoplasma capsulatum* and *Toxoplasma gondii* [53–55].

A number of not mutually exclusive factors may be involved in infection-induced thymic atrophy: fewer precursor cells may enter the thymus, these cells may have a lower ability to divide, they may die more frequently, and the rate at which they leave the thymus may be higher. The proliferative ability of thymocytes from mice acutely infected with *T. cruzi* was observed to be reduced due to lower production of interleukin-2 (IL-2), and this in turn was linked to elevated levels of IL-10 and interferon-γ (IFN-γ) [56]. There is also evidence that *T. spiralis* infection can affect the proportions of different thymocyte subsets, which is seen as a reduced responsiveness of the thymocytes to the T cell mitogen, concanavalin A. On the other hand, the proliferative response of thymocytes from *S. mansoni*-infected mice, to concanavalin A is unaffected [57]. It appears therefore that some, but not all, parasites bring about a reduction in the proliferative ability of thymocytes that could account at least in part for the 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* [58, 59] and *Plasmodium berghei* infection [51, 60]. Most of the cells that die are $CD4^+CD8^+$ thymocytes, but other subtypes such as double-negative (DN) T cells and SP cells are also reduced in number [53]. Glucocorticoid hormones appear to be responsible for the atrophy and thymocyte death during parasitic infections. In acute infections serum glucocorticoid levels rise and thymocyte caspases-8 and -9 are activated [55]. Higher levels of serum glucocorticoids have also been found in experimentally induced malaria, American trypanosomiases or Chagas disease, African trypanosomiases or sleeping sickness, toxoplasmosis, leishmaniasis, and schistosomiasis. Both tumor necrosis factor (TNF-α) and glucocorticoid serum levels rise in acute experimental *T. cruzi* infections and are associated with thymic atrophy and thymocyte depletion [53, 55, 61–64]. However additional mechanisms have been implicated, at least, in *T. cruzi* infections. Some of the factors that may be responsible for the apoptotic cell death are the *T. cruzi*-derived *trans*-sialidase [65], host-derived galectin-3 [66–69], and androgens and extracellular ATP [70].

3. Premature Release of Immature Thymocytes Bypasses Negative Selection During *Trypanosoma cruzi*-Induced Thymic Atrophy

In both murine and human models it has been shown that infection with the intracellular protozoan *Trypanosoma cruzi* results in thymic atrophy and release of undifferentiated DP T cells to periphery [61, 71, 72]. These events appear to be linked to a particular pathogen-host relationship formed during infection. In *T. cruzi* infections, the inflammatory syndrome induced by TNF-α in the acute phase of infection activates the hypothalamus-pituitary-adrenal (HPA) axis leading to release of corticosterone, and this appears to be related to the changes in both lymphoid and nonlymphoid thymus compartments and to disease outcome [59, 73, 74]. However, other host-derived molecules play a part in the thymocyte depletion. Thymic atrophy, for example, is not seen in *T. cruzi*-infected galectin-3 knockout mice [55, 66]. On the other hand, parasite-derived factors have also been shown to affect the thymic homeostasis. This is the case for the parasite *trans*-sialidase [75–77], which is implicated in the death of intrathymic T cells [78]. Moreover, the dramatic changes in the thymic microenvironment occurring 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 [72].

Until recently it was not clear whether intrathymic negative selection was affected by the alterations in the thymic microenvironment caused by *T. cruzi* infection. Clonal deletion normally appears to occur late in thymocyte development at the transition from DP to SP cells and happens at the junction between cortex and medulla [79]. In this connection, thymic metallophilic macrophages present precisely at this boundary have been shown to be involved in thymocyte maturation [80]. They differ in a number of ways from cortical macrophages and from medullary interdigitating cells and, in particular, contain large endocytic compartments devoted to the processing and presentation of antigens by

MHC class II molecules [81]. The topological distribution of these metallophilic macrophages is altered by infection; their number increases, and while some remain in the corticomedullary region, many more are spread throughout the cortex. The thymic localization of these cells may indicate that clonal selection switches from the corticomedullary junction to the cortex [82].

It is generally accepted that T cell development is controlled by interactions between TEC and thymocytes [83, 84]. Thus, when the thymic architecture is disturbed, the pattern of expression of autoantigens by TECs is altered, as well as the functioning of the thymus [85, 86]. Atrophy of the medulla and reduced levels of Aire and tissue-restricted self-antigens (TRA) transcripts are seen in mouse models deficient in a number of genes of the NFκB pathway, such as TRAF6, NIK, RelB, or p52, suggesting that this pathway is important for development of the thymic medulla [87, 88]. We measured the expression of Aire and of highly selective tissue-restricted antigens [89] by real-time PCR in whole thymuses of *T. cruzi*-infected mice and detected levels that were quite similar to control levels. These findings suggest that the expression of peripheral antigens in the infected thymuses is sufficient to modulate the induction of tolerance by negative selection [82].

Later in the course of infection, thymic atrophy becomes apparent and there is a rise in the number of apoptotic intrathymic DP T cells [90]. Although this increase may be due to the changes observed in the organ, we found that expression of Bim, a proapoptotic factor essential for negative selection [78, 91], was maintained while the number of DPs declined [82]. Furthermore, using the OTII TCR transgenic system which recognizes a $CD4^+$ epitope from the chicken ovalbumin (OVA) protein, we observed that apoptosis of TCR-stimulated semimature thymocytes was provoked when we gave the cognate OVA peptide to acutely infected mice undergoing thymic atrophy. Taken together, these data show that DP T cells can be negatively selected during infection-induced thymic atrophy, suggesting that negative selection operates normally. This is consistent with the finding that mature single-positive $CD4^+$ or $CD8^+$ T cells within the thymus do not harbor forbidden TCR genes, unlike their DP counterparts [82].

4. The *Trypanosoma cruzi trans*-Sialidase Promotes the Escape of Immature Double-Positive $CD4^+CD8^+$ Thymocytes in Chagas Disease

T. cruzi cannot synthesize sialic acid *de novo*, and the *trans*-sialidase allows the parasite to scavenge α-(2,3)-linked sialyl residues from exogenous glycoconjugates and to transfer them to acceptors resembling mucin that covers the surface of the parasite [75, 77, 92, 93]. When the sialylation profile of cell surface receptors is altered by the TS this can induce apoptosis of cells of the immune system *in vivo*, including those in the thymus [94, 95], and this apoptosis is associated with cell death in the thymic nurse cell complex [96, 97]. Several mouse thymocyte cell surface proteins that accept sialyl residues in TS-catalyzed reactions have been identified by mass spectrometry [97]. Moreover we have observed that the TS, is able to activate the c-Jun N-terminal kinases (JNK) MAPK pathway in thymocytes [98]. JNK controls the migration of a variety of cell types and cell adhesion and migration depend on cytoskeletal structures involving the actin network and actin-binding proteins, which are known to be phosphorylated by JNK [63, 99, 100]. In fact, we have shown that the cell-signaling processes involving the TS mobilize actin filaments [98], and others have shown that these signaling processes depend on interactions between TEC and thymocytes, which are required for migration of thymocytes during intrathymic development [101].

We have recently tested whether the *trans*-sialidase can itself alter the migratory responses of thymocytes, which could account for the release of DP T cells from the thymus during infection. We found that the *T. cruzi* TS increased the adhesion of thymocytes to TEC [98]. Interestingly, in order to migrate, thymocytes must adhere to their microenvironment and then dissociate from it so that molecules with adhesive and deadhesive properties may affect their migration [59, 102]. In fact, we found that treatment of thymocytes with *T. cruzi* TS *in vitro* stimulated their migration towards fibronectin, indicating a role for this enzyme in the migration of these cells [98]. The migration of thymocytes is a key event in their intrathymic differentiation [59, 103].

Our findings indicated that the migratory properties of intrathymic thymocytes are modulated by the parasite *trans*-sialidase (TS). Several steps involve migration, including entry of the precursor cells into the thymus, movement of immature thymocytes within the cortex and from there to the medulla, and, finally, exit from the thymus [59, 72]. All these events depend upon initial adhesion to the extracellular matrix (ECM) elements followed by dissociation and binding to TEC or microenvironmental components [67, 72, 104]. Interestingly, we found that *trans*-sialidase treatment of $CD4^+CD8^+$ cells within the thymus affected their maturation, resulting in a premature thymic emigration and increase in the number of recent DP thymic emigrants in the periphery, which ultimately account for the thymic atrophy process. In humans, we detected a possible correlation between the presence of the *trans*-sialidase and the number of peripheral blood DP T cells in chronic chagasic patients with the cardiac clinical forms of Chagas disease [98]. The presence of peripheral activated DP T cells with potentially autoreactive TCR may contribute to the immunopathological events found in this disease, and our data support the potential utility of chemotherapy against the parasite TS in Chagas disease.

5. Peripheral Differentiated $CD4^+CD8^+$ T Cells with Activation Phenotype Correlate with Severity of Chagas Disease

It is clear that DP T cells are released prematurely from infected thymuses into the periphery, where their maturation into functionally competent single-positive cells continues [105] even though intrathymic checkpoints remain active in the acute phase of murine Chagas disease [53, 82]. This

unconventional and rare (<5%) lymphocyte population is also seen in secondary lymph nodes, as we demonstrated in an experimental model of Chagas disease in which a DP T cell subset increased up to 16-fold in subcutaneous lymph nodes [82, 106]. Release during both acute and chronic *T. cruzi* infections may be promoted by the high level of CD62L (L-selectin), which controls lymphocyte homing to lymph nodes [107]. We have examined whether DP T cells exhibit activated properties similar to effector/memory single positive T cells [82].

Interestingly, we found that these cells do indeed develop an activated phenotype, with high levels of the activation markers CD44 and CD69, which are tightly linked to the differentiation status of T cells [82]. Moreover, when we purified (>98%) DP cell obtained by cell sorting from infected mice they were found to produce high levels of IFN-γ mRNA [82]. Furthermore, we observed that these DP T cells had higher cytotoxic activity than naïve single-positive $CD4^+$ or $CD8^+$ T cells [108, 109], in agreement with previous observations on the extrathymic DP T cells in *cynomolgus* monkeys and chimpanzees infected with hepatitis C and SIV virus [110–113], as well as in patients infected with cytomegalovirus (HCMV) and human immunodeficiency virus (HIV) [114–119]. Most significantly, patients with the cardiac form of Chagas disease had increased numbers of peripheral blood HLA-DR^+ DP T cells [82]. These findings suggest that this DP T cell subset is associated with the development of the cardiac form of the disease and probably represent activated cells [82, 106].

The studies summarized above suggest that the DP T cell subset plays a role in the inflammatory processes generated by parasite-driven immune responses. It may be that coexpression of CD4 and CD8 molecules on the T cell membrane increases the affinity of the TCR for its target cell. If so, the simultaneous triggering of CD4 and CD8 coreceptors by MHC-antigen complexes would lower the threshold for antigen signaling and reduce the need for costimulatory signals in order to achieve activation of the T cells [120, 121]. As a result, the DP T cells could be activated by low antigen concentrations and might be activated at the onset of infection when parasite-derived antigens are limiting. It could be therefore that these cells promote adaptive immune responses by secreting cytokines which could stimulate dendritic cells, so providing a link between innate and adaptive immune responses. On the other hand they might eliminate activated antigen presenting cells, since theoretically they recognize both class I and class II MHC complexes, and this would favor the infecting parasite (Figure 1).

In conclusion, despite the fact that the key intrathymic checkpoints required for negative selection are maintained during acute chagasic thymic atrophy, the peripheral DP T cells have an activated phenotype like that ascribed to activated and memory SP T cells: they produce IFN-γ, express CD44 and CD69, and have cytotoxic activity [82]. This is of special significance since fully activated peripheral DP T cells are seen in the cardiac form of chronic Chagas disease [82, 106]. The presence of these cells challenges current views concerning the T cell populations involved in adaptive immune responses during *T. cruzi* infections: it suggests that DP T cells participate from early on in the immune response against such infections. The *in vivo* role of these cells remains unclear, but the fact that they have potentially autoreactive TCR may influence the immunopathological events that occur in both murine and human Chagas disease. If it turns out that the level of DP T cells is correlated with the extent of myocardial damage in the cardiac form of the disease, these cells could act as clinical markers of disease progression and might contribute to the design of alternative treatments. These studies of the role of the DP T cells should provide fundamental insight into virus-host relationship established during *T. cruzi* infections.

6. Peripheral Activated $CD4^+CD8^+$ T Cells May Be a Common Feature in Infectious Diseases

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 acute and chronic viral infections [113, 114, 117, 118, 122–125]. Unlike immature thymic thymocytes, peripheral DP 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 [114, 125–127]. This T cell subset was associated with protection in pigs vaccinated against pseudorabies virus [126, 127]. 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 [128]. 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* [129].

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 [130, 131]. Moreover intestinal DP T cells are at least 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 [132, 133]. On the other hand circulating levels of DP T cells were not found to be reduced in HIV-1-infected patients and have even been found to be increased in some patients [114, 116]. 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 [108, 115, 117].

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 [117, 134]. 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 [135, 136]. This may be the case for HCV infections, in which the intrahepatic and circulating DP T cells are populations of activated, central, and effector memory cells, with heterogeneous differentiation patterns [109, 113]. Differences in the measured proportions of circulating DP T cells could depend on the clinical phase of HCV infection or patient populations. Interestingly, 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 [113].

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 [113]. 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-1 both *in vitro* and *in vivo* [114]. 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, and 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 infection, and a greater fraction expressed LAMP markers [114, 118, 119].

7. Concluding Remarks

DP T cells are released from the thymus along with other lymphocytes, and their number nonspecifically varies with upon infection, inflammation, and autoimmune diseases [55, 129, 137–139]. Such cells could be generated outside the thymus and/or result from *de novo* expression of CD8 or CD4 in single-positive $CD4^+$/$CD8^+$ T cells. An alternative possibility is that they arise specifically in the microenvironment of the thymus (which includes thymic epithelial cells, L-Ti-like cells, local macrophages, and DCs), under the influence of pathogens, and the consequences of infections and other physiological events causing thymic involution [53, 55, 129]. In fact the properties of extrathymic DP T cells indicate that they are closely related developmentally to conventional $CD8^+$ and $CD4^+$ T cells [82]. A key question is whether activation of the peripheral DP T cells during infections depends on interaction with the cognate peptide/MHC complex (pMHC) on antigen-presenting cells (APC). Activation of those cells, which expresses nonselected $\alpha\beta$TCR, would avoid the need for stimulation of specific T cell receptors. However it is difficult to identify the basis of the nonspecific activation of the DP T cells and its possible relevance to cellular immune response to specific pathogens because of the low frequency of DP T cells. In this connection, a correlation between numbers of DP T cell subsets and the extent of inflammatory pathology could provide a clinical marker of disease progression in pathogen infections and may facilitate the design of novel therapeutic approaches to infectious diseases.

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