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Double-negative T cells during HIV/SIV infections: Potential pinch hitters in the T cell lineup

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Abstract

Purpose of the review—This review summarizes the role of CD3+CD4–CD8– double-negative T cells, which have both regulatory and helper T cell function and may have the potential to compensate for the reduced levels of CD4+ T cells during SIV/HIV infection.

Recent findings—Double-negative (DN) T cells have been characterized in several human diseases and in murine models of autoimmunity and transplantation, where they exhibit both immunoregulatory and helper T cell-like function. During the natural nonpathogenic SIV infection of African nonhuman primates, the lack of clinical disease progression is associated with the presence of DN T cells that maintain helper T cell functions while remaining refractory to viral infection. Moreover, DN T cells may compensate for very low levels of CD4+ T cells observed in a cohort of sooty mangabeys that have been infected with SIV for over 10 years and have remained free of clinical disease manifestations associated with AIDS. These studies identify a potential for DN T cells to provide critical helper function during HIV infection.

Summary—DN T cells with some CD4+ T cell functions are associated with a nonpathogenic outcome during SIV infection and represent a potential immune therapeutic target in HIV-infected patients.

Keywords

Double-negative T cells; SIV/HIV infection; Natural hosts; Helper T cells; T regulatory cells

Introduction

Loss of CD4+ T cells is a key component of the pathology of HIV infection, leaving the immune system with insufficient numbers of helper T cells to ward off opportunistic infections and cancers. A second factor driving AIDS-associated immunopathogenesis is the increased levels of immune activation and inflammation that arise during pathogenic HIV/SIV infections (previously reviewed in [1–7]). Indeed, CD4+ regulatory T cells (Tregs) have a role in suppressing elevated levels of immune activation and are also depleted during HIV infection. As the CD4 protein is a primary entry receptor utilized by HIV, the identification of a non-CD4+ T cell subset with helper T cell and Treg function and the potential to compensate for the low levels of CD4+ T cells has significant implications for inhibiting the onset of AIDS in the face of persistent HIV replication. These characteristics are exhibited by peripheral double-negative (DN) T cells, which express the pan T cell marker CD3 but

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do not express either the CD4 or CD8 proteins. DN T cells have been described previously, with regulatory roles in autoimmunity and transplantation in murine models, and recent findings have ascribed to these cells CD4+ T cell-like functions during SIV/HIV infections. It is possible that DN T cells exist as a variety of subpopulations with multiple functions in a manner similar to CD4+ T cell subsets. This review will focus on CD3+ DN T cells that have the $\alpha\beta$ T cell receptor (TCR) and their potential roles during SIV infection of different monkey species as well as HIV infection of humans.

Function, Maturation, and TCR Signaling

DN T cells expressing the $\alpha\beta$ T cell receptor are CD3+/CD4-/CD8-, and can be found in the peripheral blood, lymph nodes and gut-associated lymphoid tissue. These mature DN T cells comprise approximately 1–5% of the T cell pool in mice as well as in humans, and an even higher percentage of T cells in some monkey species [8–12]. DN T cells have been described to have important functions in a wide range of different disease states in both mice and humans [13–24]. For example, DN T cells have exhibited a capacity to function as Tregs in murine models of diabetes [25]. In addition, DN T cells possess cytotoxic potential and can kill allogeneic as well as antigen-loaded syngeneic DCs [26], autoreactive CD8+ T cells [27], and activated allogeneic and syngeneic B cells [16]. In infectious disease mouse models, DN T cells have been shown to produce IL-17 early during pulmonary *Francisella tularensis* live vaccine strain infection in mice and also secrete IFN- γ (important for controlling intracellular bacterial growth) [15]. In humans, DN T cells play T helper roles during parasitic infection, where they have been shown to make IFN- γ , TNF- α and IL-17 as a component of the immune response to *Trypanosoma cruzi* [21]. DN T cells can also be potent suppressors of CD4 and CD8 T cells proliferation when assessed by invitro assays [9, 24]. Patients with autoimmune lymphoproliferative syndrome (ALPS) demonstrate a marked increase in DN T cell numbers [14] and show a somatic gene mutation in this T cells subset [28–30]. However, in this case it is not known whether increased DN T cells are a response to the autoimmune state and are acting as Tregs, or are contributing to the autoimmune response due to their ability to produce cytokines involved in the innate and adaptive immune responses.

The precise path of peripheral DN T cell development is not known, there are three models that one might hypothesize to explain how these cells arise and are maintained in the periphery. DN thymocytes are the DN T cell subset present in the thymus during the early stages of T cell development. These pre-T cells lack expression of the $\alpha\beta$ TCR, CD4 or CD8 and precede the double-positive stage, having not yet undergone positive or negative selection (Fig. 1). One model proposes that these immature DN thymocytes acquire expression of the $\alpha\beta$ TCR, bypass the subsequent double-positive (DP) and single-positive (SP) stages of classical T cell maturation, and migrate directly to the periphery (Fig. 1, Model 1). A second model suggests that peripheral DN T cells arise in a manner similar to single positive T cells, in which the strength and duration of signaling through the TCR complex dictates the fate of the developing thymocyte. The “strength of signal” model states that moderate TCR:MHC binding leads to the generation of single positive CD4+ and CD8+ T cells [31–33] while strong TCR:MHC binding results in apoptosis. Experimental evidence suggests that strong TCR:MHC binding that is not sufficient for induction of apoptosis may promote the conversion of DP thymocytes into DN T cells, which are able to avoid negative selection and escape from the thymic environment [8, 34, 35] (Fig 1, Model 2). These DN T cells then reach the periphery where they respond to antigen, expand and become memory cells. This model is supported by the existence of DN T cells with a memory phenotype and a polyclonal T cell repertoire [36, 37] as well as by mouse studies in which exposure of DP thymocytes to high affinity antigen leads to the generation of DN T cells via downmodulation of CD4 and CD8 [35]. The third model postulates that DN T cells arise

from mature single positive CD4⁺ T cells that have down-modulated their CD4 molecule (Fig. 1, Model 3). In African green monkeys, a proportion of peripheral CD4⁺ T cells downmodulate CD4 expression as they become antigen experienced [38]. The loss of CD4 is associated with an increase in surface expression of CD8a in these animals. It is possible that loss of the CD4 molecule might lead to the generation of DN T cells in other hosts as well. Whether DN T cells seen in the periphery of humans and nonhuman primate arose from one or more of these processes is currently under investigation.

An additional outstanding question regarding DN T cells is how TCR-mediated signaling events are induced without help from the CD4 and CD8 co-receptors that generally perform critical TCR signaling functions. In CD4⁺ T cells, the CD4 molecule facilitates antigen recognition by binding directly to MHCII on the surface of antigen presenting cells, thus stabilizing contact between the TCR and MHCII-peptide complexes [39, 40]. In the case of CD8⁺ T cells, the CD8 molecule recognizes and binds to MHCI molecules. Both CD4 and CD8 have intracellular domains responsible for associating with the protein tyrosine kinase Lck (p56) [41, 42], which is activated by autophosphorylation. Activated Lck and Fyn (associated with the TCR) then phosphorylate the immunoreceptor tyrosine-based activation motifs (ITAMs) of the TCR ζ chain and CD3 molecule [43] and initiate a cascade of events leading to nuclear translocation of NF κ -B and NFAT and transcription of genes involved in T cell function [42, 44]. The potential for antigen-specific responses by DN T cells logically requires these cells to signal through the TCR. Indeed, DN T cells from SIV-infected sooty mangabeys are capable of responding to SIV peptides [19]. One possibility is that DN T cells are able to mount TCR-elicited responses independent of a CD4 or CD8 coreceptor. Studies in mouse models have shown that the ability of CD4 to activate Lck is dispensable during CD4⁺ T cell development [45]. The consequence of this simplistic interaction may be limited breadth, and may require high-affinity TCR:peptide-MHCII interactions, as predicted by the CD4-deficient mouse model [46]. Another possibility is that DN T cells are less dependent on Lck activation [23, 47, 48]; activation of Fyn along with LAT and Erk kinases may be sufficient to activate ITAMs in DN T cells [31, 48–50]. Alternatively, DN T cells may employ an alternative TCR signaling pathway, such as the G α 11-dependent phospholipase C β -mediated pathway that is utilized by superantigens [51, 52]. Clearly, further studies of DN T cells are necessary to understand the signaling events that occur following T cell receptor binding in this T cell subset.

Role of double-negative T cells during SIV infection of natural host monkey species

Some of the most provocative studies assessing functional roles for DN T cells during lentiviral infections have come from studies of SIV-infected monkey species that are infected with SIV in the wild. Indeed, SIV is found to naturally infect at least 40 African primate species (termed ‘natural hosts’), generally resulting in no clinical signs of disease or simian AIDS [1–4, 6, 53]. The zoonotic transmission of SIV from sooty mangabeys to humans resulted in the HIV-2 epidemic (endemic to Western Africa) [54–56], as well as the SIV infection of macaques where pathogenic SIV infection was first observed [57, 58]. The absence of disease progression in SIV-infected natural host primates is neither due to low levels of viral replication (as viral loads are similar to those in pathogenic infections) [1, 2, 59–61] nor due to a superior immune response [62–64]. A number of studies have identified a lack of systemic immune activation during the chronic phase of infection to be the most significant factor inhibiting disease progression in natural hosts [2, 65–67]. In natural host monkey species two different T cell subsets have been described that lack CD4, the first is CD3⁺CD4[–]CD8^{dim} cells [12, 38] and the second is CD3⁺CD4[–]CD8[–] DN T cells [12, 19] (these are distinct from invariant chain NKT cells [68] as they have a diverse T cell receptor repertoire). Vinton et. al., performed a cross sectional analysis of DN T cells in different

natural hosts to elucidate their function and revealed that DN T cells are found in larger number (10–40% of lymphocytes) in natural hosts (sooty mangabeys, African green monkeys and patas monkeys) than seen in a non-natural host species (rhesus macaques) [12]. In addition to peripheral blood, DN T cells are also present in different immunological tissue sites including lymph nodes, lungs, and rectal mucosa [19, 61, 69, 70]. These tissue sites also maintain DN T cell numbers as CD4⁺ T cells are depleted during SIV infection [19, 61, 69, 70]. Indeed, peripheral blood assessment indicated that DN T cells are predominantly memory cells, with the majority of these cells having a central memory phenotype (expressing CD28, CD95 and CCR7). In addition, DN T cells have been shown to express CD40L, suggesting that they are able to provide B cell help, and FoxP3, suggesting regulatory function [12]. Additionally, stimulation of DN T cells from both SIV infected and uninfected natural hosts results in their secretion of IL-2 and IL-17, two cytokines generally produced by CD4⁺ T cells [12, 38]. It is therefore likely that DN T cells in natural host exist as distinct sub-populations with distinct functional attributes in much the same way that CD4 T cell subpopulations function.

We recently assessed the role of DN T cells in a cohort of SIV-infected sooty mangabeys that exhibited a dramatic CD4⁺ T cell depletion (to AIDS-defining levels) and have remained CD4-low for over 10 years without any clinical signs of AIDS [19, 61]. These CD4-low mangabeys maintain SIV-specific cytotoxic T lymphocyte and antibody responses, low levels of systemic immune activation, preserved lymphoid architecture, and preservation of other immune cell subsets [61]. Analysis of DN T cells in these CD4-low animals demonstrated that DN T cells were able to make the CD4-like cytokines IFN- γ , IL-4 and IL-17 when stimulated through their T cell receptor (anti-CD3/anti-CD28) [19]. DN T cells also responded to SIV-specific peptides as well as influenza vaccination. The DN T cells in the SIV-infected CD4-low mangabeys also maintained a central as well as effector memory phenotype throughout the course of SIV infection and, as predicted, were poor targets of SIV infection *in vivo* (likely due to the lack of CD4 expression on their surface) [12, 19]. The maintenance of DN T cell functionality during the SIV mediated CD4 T cell loss identifies a potentially key role they play in inhibiting progression to simian AIDS in natural host monkey species.

Double-negative T cells during pathogenic HIV/SIV infections

DN T cells can also be observed in Rhesus macaques, a monkey species associated with a pathogenic disease outcome following an SIV infection. In macaques, DN T cells comprise 1–5% of circulating lymphocytes and are predominantly memory cells, suggesting prior antigen exposure [12]. Macaque DN T cells are prevalent in tissues including gut-associated lymphoid tissue, indicating a potential to function at immune effector sites [69]. The functionality of macaque DN T cells has been suggested through the expression of CD40L and FoxP3 [12]. Similar to what is seen in mangabeys, macaques maintain DN T cells in peripheral blood throughout the course of SIV infection (both controlled and progressive infection) [60]. However, DN T cells in the blood were also observed to have elevated active Caspase 3 expression following SIV infection [60]. It is possible that this elevated apoptosis level seen in the DN T cells could be accompanied by elevations in production, proliferation or re-distribution of these cells in order to maintain the DN T cells during SIV infection.

DN T cell levels are also elevated in HIV⁺ patients during both the primary and chronic phases of the infection [17, 71] and recent reports have identified a regulatory role for these cells during HIV-1 infection. During primary HIV-1 infection, increased DN T cell levels were associated with lower viral load in HIV⁺ patients and the production of IL-10 and TGF- β by the DN T cells is thought to contribute towards this control [72]. In addition, increased numbers of DN T cells are associated with lower levels of CD8⁺ T cell activation

in primary HIV-1 infection further supporting a role for DN T cell as a regulatory subset [72]. These regulatory DN T cells have the potential to inhibit disease progression by limiting the levels of chronic immune activation during HIV infection. It is important to note that some researchers have identified a subpopulation of DN T cells that are HIV infected. These studies hypothesize that these DN T cells had previously expressed the CD4 protein, but had lost CD4 expression following HIV infection [73–75]. These terminally infected DN T cells would not be a sub-population that would likely play a significant role in inhibiting HIV disease progression and functionally important DN T cells likely arise from one of the models presented above (Fig. 1).

These studies of DN T cells in pathogenic HIV/SIV hosts have identified some functional characteristics that are similar to nonpathogenic SIV infections. It is interesting to speculate that DN T cells exist as a variety of subpopulations with multiple functions indicating similarities between DN T cells and the diverse subpopulations of CD4+ T cells.

Conclusion: Therapeutic intervention to augment double-negative T cell function in HIV-infected patients may improve clinical outcomes

This review highlights the potential of DN T cells to function as both immunoregulatory and helper T cells, two functions that may be important for inhibiting disease progression during HIV infection. In natural hosts that avoid progression to simian AIDS (such as sooty mangabeys and African green monkeys), it has been demonstrated that DN T cells exhibit helper T cell functions, as well as mount an SIV specific response. In contrast, DN T cells in HIV+ patients primarily display a regulatory T cell function that could be important for control of the systemic immune activation. We speculate that in HIV+ patients disease progression may be abrogated by harnessing DN T cells in two ways: first, by increasing the numbers of DN T cells, thereby enabling an expansion of their existing regulatory T cell activity, and second, by broadening the function of DN T cells to better mimic SIV+ mangabeys, thereby improving their ability to provide helper T cell function. It is therefore important to consider how one might utilize immune therapeutic approaches to improve the number and function of DN T cells in HIV-infected patients resulting in improved immune health and reduced numbers of opportunistic infections.

One candidate therapy that could promote DN T cell populations is interleukin 7 (IL-7), a cytokine involved in the homeostasis and maintenance of T cell populations [76, 77]. The presence of the IL-7 receptor (CD127) is an indicator of the capacity of T cells to respond to IL-7 and this receptor is expressed on DN T cells. Several clinical trials have been undertaken to assess the impact of IL-7 therapy on CD4+ T cell homeostasis in HIV-infected patients who do not recover CD4+ T cells despite viral suppression by antiretroviral therapy. These studies have demonstrated promising increases in both CD4+ and CD8+ T cell pools in the peripheral circulation [77–82]. In addition, several studies involving the administration of IL-7 in SIV-infected rhesus macaques have demonstrated clear and sustained increases in T cell populations in the peripheral blood [83–87], including the naïve and central memory CD4+ T cell compartments [76, 88]. Of note, there were no marked increases in plasma viremia in SIV-infected rhesus macaques receiving IL-7 [89]. Two other T cell homeostatic cytokines, IL-2 and IL-15, have also been studied extensively as candidate therapeutics during SIV/HIV infections [90–93]. To date, however, studies of homeostatic cytokines in HIV-infected patients and SIV-infected macaques have not examined the impact of cytokine therapy on DN T cell number and function.

The differences between DN T cell function during pathogenic HIV/SIV infection and non-pathogenic infections may be attributable to the generalized immune dysfunction seen in pathogenic infections. The studies reviewed here identify DN T cells as a potential target for

a future immune therapeutic (utilizing IL-7 or another immune modulatory cytokine), with the goal of increasing the levels and/or function of a DN T cell population that would be refractory to HIV infection yet provide critical helper and regulatory function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key findings

- In humans and mouse models of autoimmunity and infectious diseases, double-negative (DN) T cells display functions characteristic of T helper and T regulatory cells.
- DN T cells predominately have a memory phenotype, are polyclonal and are found in the peripheral blood and lymphoid tissues of humans and non-human primates.
- In SIV infection of natural hosts, DN T cells may be able to compensate for CD4 T cells loss due to SIV infection as they exhibit CD4 helper T cell functionality.
- DN T cells are maintained throughout the course of SIV infection of Rhesus macaques, and in primary HIV infection DN T cells are associated with decreased viral load and CD8 T cell activation.

Figure 1. Origin of double-negative T cells

Immature thymocytes undergo a stepwise maturation (shown in block arrows) in the thymus, beginning with the rearrangement of the alpha-beta (ab) T cell receptor (TCR) yielding a double-negative thymocyte with a pre abTCR that is lacking CD4 and CD8 expression. They then undergo rapid proliferation accompanied by the upregulation of both CD4 and CD8, as well as low expression of CD3. As these double-positive thymocytes undergo positive selection for TCRs able to recognize self MHC, they downmodulate CD4 and CD8 transiently. Negative selection then removes the T cells that react too strongly to self MHC:peptide by apoptosis. A moderate TCR:MHC binding yields single positive CD4⁺ and CD8⁺ T cells which migrate to the periphery. Mature DN T cells may reach the periphery by three possible ways shown by dotted arrows. The first model is that immature DN thymocytes acquire expression of the abTCR, bypass the single positive stage of maturation, and migrate to the periphery (1). The second model proposes that during negative selection a stronger TCR:MHC binding allows the transient DN thymocyte to escape to the periphery and to further encounter antigen as functional DN T cells (2). The third model is that DN T cells originate after the generation of single positive CD4⁺ T cells due to CD4 downmodulation in the periphery (3).