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Double-negative T cells in autoimmune diseases

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Abstract

Purpose of review—TCRα^{+}CD4^{-}CD8^{-} double negative T (DNT) cells, a principal subset of mature T lymphocytes, have been closely linked with autoimmune/inflammatory conditions. However, controversy persists regarding their ontogeny and function. Here we present an overview on DN T cells in different autoimmune diseases to advance a deeper understanding of the contribution of this population to disease pathogenesis.

Recent findings—DNT cells have been characterized in various chronic inflammatory diseases and they have been proposed to display pathogenic or regulatory function. The tissue location of DN T cells and the effector cytokines they produce bespeak to their active involvement in chronic inflammatory diseases.

Summary—By producing various cytokines, expanded DNT cells in inflamed tissues contribute to the pathogenesis of a variety of autoimmune inflammatory diseases. However, it is unclear whether this population represents a stable lineage consisting of different subsets similar to CD4 T helper cell subset. Better understanding of the possible heterogeneity and plasticity of DNT cells is needed to reveal interventional therapeutic opportunities.

Keywords

Double negative T cells; ontogeny; heterogeneity; autoimmune diseases

Introduction

The most important hallmark of immune disorders is the activation and accumulation of T lymphocytes, the majority of which express both alpha and beta chains of the T cell receptor (TCR) and are therefore referred as αβ T cells[1]. Among αβ T cells, CD4^{+} helper or CD8^{+} cytotoxic T cells are most prevalent subsets[2]. However, a small population of αβ T cells which do not express both CD4 and CD8, termed “double negative” T (DNT) cells[3,4], have been considered to contribute to the pathophysiology of a series of autoimmune diseases[4].

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Conflicts of interest

There are no conflicts of interest.

DN T cells were initially identified and characterized in *lpr* and *gld* mice (deficiency of either Fas or Fas ligand) in which lymphoproliferative syndrome developed due to impaired Fas-mediated apoptosis[5–9]. The massively expanded DNT cells results in the lymphadenopathy and splenomegaly which leads to the early hypothesis that DNT cells are immunopathogenic[5]. Later on, expanded DNT cells were observed in patients with different immune disorders including Autoimmune lymphoproliferative syndrome (ALPS) [10,11], systemic lupus erythematosus (SLE)[12,13] and sjogren's syndrome[14,15]. Although DN T cells only represent a small portion of αβ T cells compared to either CD4 or CD8 T cells in normal subjects[16,5], the expansion of DN T cells in various autoimmune diseases and the presence of DNT cells at sites of injury in different inflammatory conditions strongly suggest their critical roles in inflammation[4]. However, our understanding of DNT cell ontogeny and function still remains limited[17,3–5].

We propose that the discrepancy on the differentiation and function of DNT cells could be explained by the heterogeneity and plasticity of this type of cells.

Ontogeny of DNT cells

In healthy individuals, DNT cell only comprise a small portion of αβ T cells and are considered quiescent[5,4]. αβ T cells are derived from the developing progenitors within the thymus, the thymocytes. Developing thymocytes undergo a series of maturation steps before egressing from the thymus[18] and the earliest developing thymocytes lack the expression of the co-receptors CD4 and CD8 and are termed double negative (DN) population[18,19], which leads to the hypothesis that peripheral DNT cells may represent primitive αβ T cells which originate in the thymus but escape the late development followed by migration to the periphery (Fig. 1a)[20,3]. For late stage thymocyte development, TCR signal strength and duration determine the lineage commitment to either $CD4^+$ or $CD8^+$ T cells. Typically lower intensity TCR signals leads to full maturation of either $CD4^+$ or $CD8^+$ T cells while cells with high TCR strength are deleted during the development to avoid autoimmunity[21,22]. This process has be well recapitulated by *in vitro* cultured thymocytes in the presence of cortical epithelial cells[23]. Considering the fact that CD4 or CD8 expression is essential in augmenting TCR signaling by stabilizing interactions between TCR-MHC complex[24,25], it is reasonable to postulate that low or negative expression of CD4 and CD8 coreceptors protects thymocytes away from high intensity TCR signaling mediated depletion and promotes their thymic egress[26,27]. In contrast to low concentrations of ligands that induce maturation to single positive (SP) thymocyte, double positive (DP) thymocytes cocultured with cortical epithelial cells loaded with high concentrations of high affinity ligands acquire DNT phenotype with downregulation of both CD4 and CD8[20,28].

However, there is sufficient evidence to suggest that DNT cells are generated in the periphery. For example, DNT cells can develop in thymectomized mice reconstituted with T cell-depleted bone marrow cells[29]. The fact that mice deficient in β2-microglobulin have reduced DNT cell lymphoproliferation[30] and polyclonal DNT cells regain CD8 expression in lymphopenic environment[31], indicates that DNT may derive from peripheral mature CD8 T cells[4]. Similar evidence was generated from human studies[32,33]. First, gene expression pattern analysis revealed that DNT display more similarities with CD8 rather

than CD4 T cells[32]. Second, the analysis of Vα and β usage of TCR revealed the high similarity between CD8 T and DNT in patients with ALPS[34]. The dysregulated DNT cell homeostasis in *lpr, gld* mice and ALPS patients[35–37] has directed the attention to defective apoptosis mediated by Fas dependent pathway[38,39]. The loss-of-function mutations in the Fas pathway in T cells lead to impaired apoptosis after repeated TCR engagement[9,11]. Activation-induced cell death (AICD), a Fas/FasL dependent negative regulator of activated T cells upon repeated TCR stimulation[40,41], is important for the maintenance of T-cell homeostasis and abnormalities in this process may result in autoimmunity[42]. The evidence above depicts a possible model for the pathogenic DNT cell expansion in autoimmunity in which autoreactive CD8 T cells skip antigen induced AICD by losing CD8, and execute their pathogenic role *in vivo*[5,4]. Along this line, TCR$\alpha\beta^+$ CD8 T cells lost their CD8 expression upon stimulation with high concentration of anti-CD3 *in vitro*[32,33]. Adoptively transferred CD8 T cells with transgenic TCR acquired DNT like phenotype after encountering exogenous or endogenous antigens *in vivo*[43,44,16]. Moreover, increased Ki67 expression, narrowed TCR Vβ repertoire usage and diluted T-cell receptor excision circles (TRECs) observed in DNT cells indicated the clonal proliferation and expansion possibly driven by endogenous self-antigens[44–49]. Of note, *in vivo* antigen administration to MHC class I-restricted TCR transgenic mice on *lpr* background resulted in expansion of DNT cells[33], which further supported the concept that the expanded DNT cells under chronic inflammation might derive from antigen activated CD8 T cells[4,17]. However, it remains a mystery whether the absence of proper apoptotic signals or addition of supportive signals such as cytokines help activated CD8 T cells escape AICD and acquire DNT cell phenotype (Fig. 1b).

To date, the controversy on the origin of DNT cells continues. There are several possible scenarios which are worth of attention: (1) DN T cells directly originate from those immature DN thymocytes which could not recognize MHC class I or MHC class II molecules but for some reason are not appropriately depleted in thymic positive selection. (2) DN T cells represent a unique lineage which is selected by recognition of neither class I nor MHC class II but certain unknown MHC like molecules. (3) There are different types of DNT cells with either intrathymic or extrathymic origin, a model which we favor most since it fits the best for the above augments [17,5].

DN T cells, the evil or the angel in inflammation

Under naïve status, DNT cells represent a minor population in total $\alpha\beta$ T cells with unrecognized roles in immune system. However, the lupus like symptoms in *lpr* or *gld* mice and disease-associated expansion of DNT cells lead to the supposition that DNT cells are assigned a pro-inflammatory role[50,9,6–8]. The findings that DNT cells are also expanded in patients with various inflammatory rheumatic disorders including ALPS and SLE reinforce this concept[12,11]. Evidence has emerged which supports the pathogenic role of DNT cells[4]. *Ex vivo* analysis on the cytokine profiles of DNT cells from various murine models has shown the great ability to produce various inflammatory cytokines including IL-2, IL-4, TNFα and IL-17A[43,31,51]. Similar results were also achieved from studies in human subjects with diverse autoimmune diseases[4]. In addition, DNT cells provide help to B cells to enhance autoantibody production *in vitro*[12]. Immune cell infiltration is generally

considered as a major contributor of tissue damages during chronic inflammation[52]. Along this line, DNT cells are present in inflamed kidney[13,44,48,47], skin[53], salivary gland[14], entheses[54] and ischemic brain[55], which suggests they present good therapeutic targets to control inflammation in various diseases.

The activation of T cells requires signaling through TCR and the coreceptors CD4 and CD8 are essential augmenting TCR signaling[1,24,56,25]. It has been argued that the cognate TCR-antigen interaction without proper augmentation by CD4 and CD8 molecules is sufficient to drive DNT cell activation *in vivo*. Mice with concomitant deficiency of both *CD4* and *CD8* developed inflammatory responses and immunopathology compared to wild type mice during acute Staphylococcal enterotoxin B infection (SEB)[57]. Of note, chronic exposure to SEB precipitated a lupus-like inflammatory disease characterized by lympho-monocytic infiltration in multiple tissues along with production of autoantibodies in these double gene deficient mice[57]. Interestingly, disease development was accompanied with the expansion of DNT cells[57]. In line with their response to SEB, in the lung of mice challenged with live vaccine strain (LSV) of Francisella tularensis, DNT cells represent the major responding T cell subset[58]. Also in HIV-infected patients, DNT cells represent a significant portion of the cellular viral load in T cells[59,60], which suggest *in vivo* they might function similar to CD4 T cells.

In contrast to above studies, evidence has been generated suggesting that at least subsets of DNT cells exert regulatory activity[17,46]. In skin or bone marrow allograft murine model, DNT cells were capable of suppressing syngeneic CD4 or CD8 T cells in both Fas-dependent and Fas-independent manners[17]. In addition, DNT cells were also capable of inhibiting NK cell-mediated rejection of allogenic bone marrow through perforin-dependent killing[61]. In agreement with their role in transplantation, a number of studies in autoimmune diabetes revealed that transferred DNT cells can efficiently prevent diabetes onset in non-obese diabetic (NOD) mice by producing IL-10[62,63,17]. The phenotypic counterparts of murine suppressive DNT cells have been identified also in humans[63,46]. Interestingly, in a small cohort of patients with allogeneic bone marrow transplantation, there was an inverse correlation between the frequency of circulating DN T cells and the severity of graft versus host diseases[64] although further mechanistic studies are needed.

Heterogeneity and possible plasticity of DNT cells

Variable phenotypes of DN T cells with diverse cytokine profiles have been reported[4,17], which indicates that DN T cells, similar as CD4 helper T cells[65,66], may be divided into different subsets. Five major CD4 helper T cell lineages, Th1, Th2, Th17, Tfh and Treg have been identified based on the expression of specific transcription factors and cytokine profile essential for fate determination and function[66,67]. DNT cells represent a relatively small population among total $CD3^+$ T lymphocytes with polyclonal repertoire[44,34,45] but they are selectively expanded under various inflammatory conditions. Of note, expanded DNT cells display many terminal differentiation characteristics including Ki67 expression, a narrowed TCR Vβ repertoire and a low content of TRECs[44,47,48,46]. In different disease models, DNT cells exhibit completely divergent cytokine profiles. For instance, in lupus and chronic infection settings, DNT cells produce pro-inflammatory cytokine IL-17, which plays

an essential role in the clearance of extracellular pathogens but also contributes heavily to inflammation mediated tissue damages. Moreover, in lupus prone mice and SLE patients, DNT cells can be sub-grouped based on PD1 expression[43]. Notably, $PD1^+$ but not $PD1^-$ DNT cells contain a large portion subset with self-reactive TCRs and they are the main source of IL-17[43], which is the first solid evidence of heterogeneity among DNT cells. Similar as Th17s[68], IL-23 promotes but IL-2 attenuates IL-17 producing DNT cells[69,44,70].

In contrast, in allograft rejection and non-obese diabetes, DNT cells produce high amounts of immunosuppressive IL-10, which is essential for their regulatory capacity[17,62,46]. Successful identification of bonafide markers to separate functionally distinct DNT subsets will help reconcile the observed discrepancies. It is possible to postulate that under naïve status, the regulatory DNT cells are predominant and essential for self-tolerance. During chronic inflammation, the balance of regulatory DNT cells with proinflammatory DNT cells is disturbed and pathogenic DNT cells characterized by IL-17 or other proinflammatory cytokine production become prevalent instead[67]. Although evidence suggests that DNT cells display a terminal differentiation status and proliferate poorly upon anti-CD3 stimulation[4], the possibility can not be excluded that DNT cells are plastic. The de novo generation of DN T cells from $CD8^+$ T lymphocytes[44,43,32] and the observation that DNT cells regain CD8 expression in lymphopenic environments pinpoint cell plasticity at least between DNT and CD8 T lineages[31]. Moreover, the key factors controlling the transition between different CD4 helper T subsets are various combinations of cytokines, which suppress or reinforce lineage specific transcription factors[67]. Considering the fact that reduction of TGFβ and increase of IL-23 create a milieu which favors the expansion of IL-17 producing DNT cells[44], the cytokine environment appears to tightly control the pathogenesis of DNT cells in chronic inflammation. It is highly possible that DNT cells, similar as their CD4 counterparts, are relatively unstable and reshaped cytokine environment may result in the fate plasticity with potential ability to switch between anti- and pro-inflammatory phenotypes, although more evidence is needed to support this postulate.

In addition, cell plasticity relies on cell heterogeneity. DNT cell pool might not represent a “pure” differentiating population. Some of them might be fully differentiated with limited plasticity, whereas others may retain the flexibility because of their partial differentiation state. Exploring the key factors controlling the redifferentiation holds the promise for future treatment of DNT cell involved inflammatory diseases.

DNT cells in autoimmune disorders

Autoimmune lymphoproliferative syndrome (ALPS) and ALPS-like diseases—

ALPS is an autoimmune disorder with a progressive lymphoproliferation, massive lymphadenopathy and splenomegaly[71,50], phenotypically similar to the autoimmunity predisposed *lpr* and *gld* mice[6,8]. The massive accumulation of DN T cells in the blood and secondary lymphoid organs, the main manifestation of chronic nonmalignant lymphoproliferation, now is considered a key requirement for ALPS diagnosis[71–73], and this elevation results from a primary defect in Fas-mediated apoptosis[72,9,41,11]. In patients who develop some features of ALPS but do not fulfill the diagnostic criteria for

ALPS, mutations in other components of pathways central to lymphocyte growth, activation and apoptosis have been identified including Caspase-8 and FADD[74,71]. These have been grouped into ALPS-like diseases and some patients in this category have increased DNT cells also[74,71].

Interestingly, CDR3 sequencing has revealed a significant overlap of TCR Vβ-Jβ transcripts between DNT cells and CD8 T cells from ALPS patients[34,49], which strongly suggest the at least partial CD8 origin of DNT cells in ALPS. The concept that DNT cells contribute to autoimmune symptoms in ALPS patients and autoimmunity predisposed *lpr* and *gld* mice comes from the following facts: (1) The progressive expansion of DNT cells is closely associated with disease development[75]. (2) The presence of autoantibodies in most ALPS patients correlates with the number of DN T cells[76,77]. (3) Effective treatment ameliorates autoimmune symptoms in ALPS with significant elimination of abnormal DNT cells[78–80]. Although the elevation of DNT cells in ALPS is not in dispute, further evidence is needed to validate their pathophysiological significance.

Systemic lupus erythematosus (SLE)—SLE is a clinically heterogeneous autoimmune disease with systemic inflammation and organ damage[81]. Various T cell abnormalities were reported and the expansion of DNT cells represents a prominent one[12,13,82]. The early observation that SLE patients have expanded numbers of DNT cells in the peripheral blood and this expansion correlates with disease activity leads to the supposition that expanded DNT cells contribute to the pathophysiology of SLE[83,13]. The first evidence was from *in vitro* co-culture assays which clearly demonstrated that DNT cells provide help to B cells to promote antibody production[12]. IL-17A, a pro-inflammatory cytokine, has been documented with crucial contribution for systemic inflammation and tissue damage in SLE[84–86,13]. The findings that DNT cells represent a major source of IL-17A in SLE patients reinforced the concept on DNT cell pathogenesis in SLE[13,4]. Moreover, DNT cell invasion in the kidneys of patients with lupus nephritis has also been recorded[37]. A series of experiments reported from our laboratory have demonstrated that a large portion of expanded DN T cells in SLE were derived from self-reactive CD8 T cells[44,32,16,31]. Self-antigens derived from apoptotic cells can activate self-reactive CD8 T cells, which give rise to DN T cells through the downregulation of CD8 expression on the cell surface. These cells displayed acquired proliferating or proliferated phenotype (Ki67 expression, diluted TREC, and narrowed TCR repertoire)[44]. CD8 Treg cells have been described as CD8 T cells specific for antigen delivered to immune-privileged sites and to control the effector T-cell responses by CD8 and perforin dependent killing[87–89]. The whole process of conversion from CD8 T cells into DNT cells contributes to the pathogenesis of lupus based on the loss of CD8-dependent immunosuppressive potentials and the acquisition of ability to produce different pro-inflammatory cytokines and especially IL-17[44]. In addition to TCR signaling and co-receptor signaling, cytokines provide the third signal for T cell activation and surviving[90]. The fact that skewed inflammatory cytokine environment in lupus favors the expansion of DNT cells suggests that cytokines compensate reduced TCR signaling strength due to the loss of CD8 for cell activation and survival[44].

Sjögren's syndrome, Psoriasis, Axial spondylarthritis and other rheumatic diseases—Sjögren's syndrome is a systemic autoimmune disease characterized by lymphocytic infiltration in salivary and tear glands[91]. Sjögren's syndrome may occur as primary disease but most often occurs in the context of other autoimmune disorders[91], including SLE and rheumatoid arthritis. Similar as in SLE, DNT cells are expanded and become the main source of IL-17 in patients with primary Sjögren's syndrome[92,14,15]. The expansion of DNT cells correlates well with disease activity and IL-17$^+$ DNT cell infiltration was detected in inflamed salivary glands[14,15].

Psoriasis is a complex inflammatory skin disease characterized by immune cell infiltration to the skin[93]. IL-17-producing DNT cell infiltration was found in the epidermis of mice with induced psoriasis[94] and patients with plaque-type psoriasis[53]. Axial spondylarthritis is another chronic inflammatory disease which affects primarily the spine and the sacroiliac joints[95] but shares many genetic features with psoriasis[96]. Interestingly, in a widely accepted murine model of spondyloarthropathy, IL-23R$^+$ DNT cells were detected in the inflamed entheses[54]. Again, these observations reinforce the perception that DNT cells contribute heavily to pathogenesis of many inflammatory rheumatoid disorders. Furthermore, DNT cells are expanded in a subset of pediatric patients with various autoimmune diseases including mixed connective tissue disease (MCTD), juvenile idiopathic arthritis (JIA) juvenile dermatomyositis[97] and Behcet's disease[98] although additional investigations are required for their precise role in these patients.

Type 1 diabetes (T1D)—Type 1 diabetes (T1D) is an organ-specific autoimmune disease with severe loss of pancreatic β cells[99]. Both CD4 and CD8 T cells play distinct and highly pathogenic roles in β cell destruction[100]. In contrast to the pathogenesis of DNT cells in inflammatory rheumatoid disorders listed above, a number of studies have demonstrated the immunosuppressive ability of DNT cells and their ability to inhibit the development of autoimmune diabetes[17,3]. First, a progressive loss of DNT cells with age was observed in non-obese diabetic (NOD) mice[62]. Second, adoptive transfer of DNT cells efficiently inhibited the development of autoimmune diabetes in several different diabetic mouse models[63,101,102]. Third, transfer of NOD CD8 T cells resulted in diabetes but co-transfer of NOD CD8 T cells with DNT cells did not, which indicates that DNT cells act directly on pathogenic T cells to exercise theirs immunosuppressive function[62,17]. However, controversies remain on the nature of immunosuppression of DNT cells. Distinct mechanisms with different molecules involved have been proposed for DNT cell mediated suppression including elimination of effector T cells by either Fas/FasL-mediated apoptosis[103,104] or perforin mediated killing[102,105,106,46] and modulation on antigen presenting cells by producing IL-10[63,62] or IFNγ[107,46,108]. Of note, both IL-10 and IFNγ function as a double-edged sword in autoimmune diseases[109,110] and the immune environments determine whether they are beneficial or detrimental. Therefore, more mechanistic studies are needed.

Therapeutic interventions targeting DNT cells

In autoimmune diseases where expanded DNT cells display distinct pathogenic capacity their selective ablation or specific modulation of the processes that render them less

pathogenic should be considered for therapeutic purposes. More attention should be given to the design of specific drugs able to limit the expansion pathogenic DNT cells or if possible favor regulatory DNT activation. In light of understanding of DNT cell generation in lupus, more and more approaches directly or indirectly targeting DNT cells have been tested. In lupus prone mice and SLE patients, a large portion of DNT cells were derived from antigen stimulated CD8 T cells. The activation induced chromatin remodeling and epigenetic silencing on various promoters and enhancers of *Cd8* locus might be responsible for the de novo generation of DNT cells from CD8 T cells. As expected, the methylome of DNT cells affirmed hypermethylation on regulatory elements of *Cd8* locus[111]. In brief, the transcription factor cAMP responsive element modulator (CREM)α orchestrates DNA methyltransferase (DNMT)3a and histone methyltransferase G9a to directly enhance DNA and histone methylation on *Cd8* locus[112,113], which results in stable epigenetic silencing[113,114]. Accordingly, genetic deficiency of *Crem* in lupus prone mice significantly ameliorates disease manifestations by reducing IL-17$^+$ DNT cells[115]. DNA methylation patterns in SLE T cells are complex with both hypomethylated and hypermethylated cytosine-guanine sites[116,117]. Generalized DNA hypomethylation in CD4 T cells has been well linked to the disease manifestation[118,119]. Surprisingly, in contrast to systemic delivery of 5-azacytidine[120], a DNA methyltransferase inhibitor, which profoundly augments disease progression[121], its targeted delivery to CD8 T cells using a nanolipogel delivery system significantly ameliorated disease severity in lupus prone mice by restraining the expansion of pathogenic DNT cells[122]. This result is consistent with the proposition that CD8 T cells acquire pro-inflammatory DNT cell phenotype through enhanced DNA methylation mediated CD8 loss[4]. In line with these observations, well controlled CD8 expression on CD8 T cells and DNT cells by proper modulation of epigenetic modification on *Cd8* locus should present a valuable therapeutic strategy for the treatment of autoimmune disease with involvement of DNT cells (Fig. 2a).

The expanded DNT cells in human and mice with defective Fas-mediated pathway depicted another picture, in which DN T cells were derived from mature T cells with failed apoptosis[5]. Along this line, DNT cells with resistance to AICD could be generated *in vitro* from Fas-sufficient T-cells with repeated anti-CD3 stimulation[33,32]. However, further studies are warranted to validate whether addition of FasL or other apoptosis inducing molecules could modulate the generation of DNT cells as expected *in vitro* and *in vivo* (Fig. 2b) since the controversies persist on the therapeutic values of FasL in autoimmune disease[123]. Fas and FasL play essential immunosuppressive roles in controlling T cell homeostasis, as recorded with the development of autoimmunity in *lpr* or *gld* mice[5]. Paradoxically, Fas also plays a proinflammatory role in certain settings since *lpr* or *gld* mice are resistant to induced rheumatoid arthritis[124] and type I diabetes[125]. The constitutive expression of Fas in many types of cells may explain the observed complexity of Fas-mediated immune response[126]. Therefore, further insights into Fas-dependent and Fas-independent DNT cell homeostasis are needed for better therapeutic strategies.

The requirements for signal 3 provided by cytokines to DNT cell activation and differentiation link the cytokine milieu to loss of CD8 expression in CD8$^+$ T cells[90,44]. It has been reported that IL-4-induced STAT6 orchestrates GATA3 for transcriptional repression of *Cd8*[114]. Interestingly, IFN-γ partially recovered CD8 expression in a subset

of DN T cells[114] which is consistent with the observation that DNT cells could re-attain CD8 expression in the proper cytokine milieu in lymphopenic hosts[31]. Furthermore, *in vivo*, elevated IL-23 along with reduced TGFβ facilitate self-reactive DN T-cell activation, expansion, and survival[44]. Targeting cytokines, specific intracellular kinases or transcription factors provides an alternative therapeutic choice (Fig. 2c) although caution has to be applied because of shared components between different pathways.

It has become clear that metabolic processes control the fate decision of T cell differentiation and further the function of T cells. In autoimmune diseases, the disturbed or skewed metabolic pathways in T cells have been frequently reported[127,128]. However, most studies focus on CD4 T cells and very little attention has been given to DNT cells. Observation made in a clinical trial of sirolimus in patients with active SLE showed dramatic reduction of IL-4^+ and IL-17^+ producing DNT cells 12 months after treatment[129], which strongly suggests that mTOR blockade corrects pro-inflammatory DNT cell differentiation and activation. Consistently, PP2A, a serine/threonine phosphatase, plays a key role in restraining the activation of the metabolic checkpoint kinase mTOR and the PP2A activating molecule FTY720 induced DNT cell apoptosis in lupus prone murine[130]. Thus, the development of novel therapies to control the activity of metabolic enzymes in DNT cells represents a promising exercise for treatment of autoimmune diseases (Fig. 2d).

Conclusion

DNT cells represent important component of the immune system[5]. Although the possibility can not be excluded that some DNT cells are direct thymic escapes, a great portion of DNT cells are generated from peripheral CD8 T cells which lose CD8 expression on cell surface following the stimulation with combination of various signals including TCR engagement and cytokine stimulation[32,43,44,17]. Distinct epigenetic processes are responsible for this process and more studies are wanted for more details[4]. The fact that DNT cells infiltrate various inflamed organs including the skin and the kidney in different diseases[4] along with their ability to help B cells to produce autoantibody[12] and various pro-inflammatory cytokines including IL-17[13] underwrites their important contribution to the pathogenesis of autoimmune diseases. It is highly possible that a subset of DNT cells may instead have regulatory capacity in certain disease settings like organ transplantation and non-obese diabetes[17]. A growing understanding of DNT cell origin and functional features has prompted the consideration of therapeutic approaches including targeted reopening of the CD8 locus, precise modulation of cell activation and survival, inhibition of proinflammatory metabolic pathways and blockade of the inflammatory milieu which enables their generation or enabling their demise.

A number of questions needs urgent attention. A clear characterization of DNT subsets is needed through novel spectral cytometry and single cell sequencing technologies. The factors which enable the expansion of proinflammatory or regulatory DNT cells in various diseases need to be defined. Using advanced protocols, including Slide-Seq[131], the exact interaction between DNT cells and other immune cells or tissue resident cells should be defined. Prospective clinical studies are needed to define their appearance during the

evolution of the disease process. Such studies may reveal that certain characteristic of DNT cells in the periphery can serve as biomarkers of organ inflammation and disease activity.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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Key bullet points

- DNT cells are expanded in various chronic inflammatory diseases and they display pathogenic or regulatory function.
- DNT cells are present in inflamed tissues and produce effector cytokines through which they exercise their function.
- It is unclear whether they represent a distinct lineage or they originate from single positive cells, whether they represent a homogenous group of cells and whether they display plasticity.

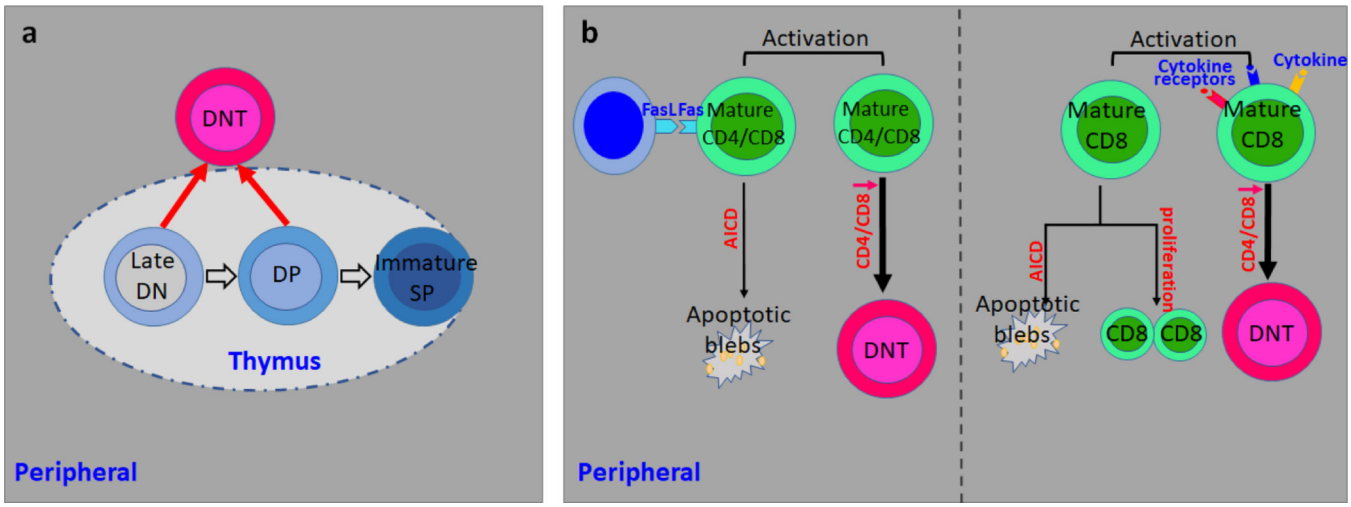

Fig. 1. Ontogeny of DN T cells.

a. Peripheral DNT cells derive directly from immature DN thymocytes or from DP thymocytes through the downregulation of both CD4 and CD8.

b. Left: Activated CD8 T cells without proper apoptotic signals escape AICD and give rise to DNT cells. Right: Cytokine signal inputs help the conversion from CD8 T cells to DNT cells.

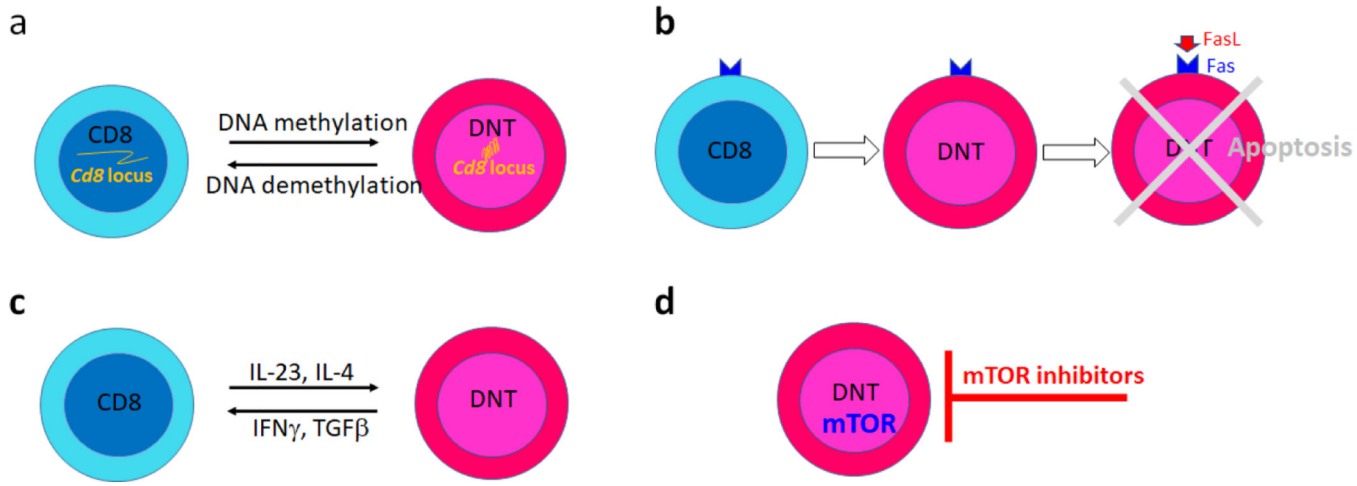

Fig. 2. Therapeutic interventions targeting DNT cells

a. Regulate the conversion between DNT cells and CD8 T cells through epigenetic modulation.

b. Eliminate DNT cells by adding missing signals for apoptosis.

c. Regulate the conversion between DNT cells and CD8 T cells by reshaping the cytokine milieu.

d. Inhibit DNT cell activation and expansion by targeting DNT cell metabolism.