



HAL
open science

CD3CD4CD8 (double negative) T cells: saviours or villains of the immune response?

Fulvio d'Acquisto, Tessa Crompton

► **To cite this version:**

Fulvio d'Acquisto, Tessa Crompton. CD3CD4CD8 (double negative) T cells: saviours or villains of the immune response?. *Biochemical Pharmacology*, 2011, 82 (4), pp.333. 10.1016/j.bcp.2011.05.019 . hal-00718034

HAL Id: hal-00718034

<https://hal.science/hal-00718034>

Submitted on 16 Jul 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: CD3⁺CD4⁻CD8⁻ (double negative) T cells: saviours or villains of the immune response?

Authors: Fulvio D'Acquisto, Tessa Crompton

PII: S0006-2952(11)00332-7
DOI: doi:10.1016/j.bcp.2011.05.019
Reference: BCP 10911

To appear in: *BCP*

Received date: 15-3-2011
Revised date: 12-5-2011
Accepted date: 17-5-2011

Please cite this article as: D'Acquisto F, Crompton T, CD3⁺CD4⁻CD8⁻ (double negative) T cells: saviours or villains of the immune response?, *Biochemical Pharmacology* (2010), doi:10.1016/j.bcp.2011.05.019

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



1
2 **CD3⁺CD4⁻CD8⁻ (double negative) T cells: saviours or villains of the immune**
3
4 **response?**
5
6
7
8

9
10 Fulvio D'Acquisto¹ and Tessa Crompton²
11
12
13
14
15
16

17 ¹William Harvey Research Institute, Barts and the London School of Medicine, Queen Mary
18 University of London, Charterhouse Square, London EC1M 6BQ, UK; ²Immunobiology Unit,
19
20 UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK
21
22
23
24
25
26
27
28

29 **Running title:** Pathophysiological role CD4⁻CD8⁻ double negative T cells
30
31
32
33
34
35

36 **Key words:** Thymocyte positive and negative selection; CD4⁻CD8⁻ DN T cells; unconventional T
37 cells; inflammation; autoimmune and infectious diseases.
38
39
40
41
42
43
44
45

46 **Correspondence:** F. D'Acquisto, The William Harvey Research Institute, Barts and the London
47 School of Medicine, Charterhouse Square, London EC1M 6BQ, UK. Tel: 44 207 882 6081. Fax:
48
49 44 207 882 6076. E-mail: F.Dacquisto@qmul.ac.uk.
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Abstract

Recent studies have shown that T cells are not just the latecomers in inflammation but might also play a key role in the early phase of this response. In this context, a number of T cell subsets including NKT cells, mucosal-associated invariant T cells and γ/δ T cells have been shown, together with classical innate immune cells, to contribute significantly to the development and establishment of acute and chronic inflammatory diseases. In this commentary we will focus our attention on a somewhat neglected class of T cells called $CD3^+CD4^-CD8^-$ double negative T cells and on their role in inflammation and autoimmunity. We will summarize the most recent views on their origin at the thymic and peripheral levels as well as their tissue localization in immune and non-lymphoid organs. We will then outline their potential pathogenic role in autoimmunity as well as their homeostatic role in suppressing excessive immune responses deleterious to the host. Finally, we will discuss the potential therapeutic benefits or disadvantages of targeting $CD3^+CD4^-CD8^-$ double negative T cells for the treatment of autoimmune disease. We hope that this overview will shed some light on the function of these immune cells and attract the interest of investigators aiming at the design of novel therapeutic approaches for the treatment of autoimmune and inflammatory conditions.

Introduction

One of the most important hallmarks of the inflammatory response is the migration and infiltration of leukocytes to the site of injury or infection [1, 2]. The identification of unique markers for the continuously growing number of cell types of the immune system has provided scientists with a wealth of information regarding the specific contribution of different subtypes of leukocytes to a variety of immune and inflammatory reactions. It is in fact possible these days to dissect out, isolate and characterize in great detail the types of leukocytes infiltrated in inflamed tissues based on the pattern of expression of their cell-surface markers.

However, the more we know about these cells the more we realize that these simple classifications are imperfect and that leukocytes can disguise themselves in many different ways. For instance, T cells have been typically identified based on the expression of the alpha and beta chains of the TCR ($\alpha\beta$ TCR) and further classified into helper or cytotoxic T cell populations based on the expression of CD4 or CD8, respectively. Interestingly, recent studies have shown that lymphocytes can also “adsorb” (or steal) classical antigen-presenting cell surface molecules through a process called “trogocytosis” (from the ancient Greek trogo, meaning “gnaw”). In the case of CD8⁺ T cells, acquisition of cognate MHC class I ligands induces cytotoxic T lymphocytes to “fratricide” antigen-specific cytolysis, thereby contributing to the clearance of CD8 [3, 4]. If the readers think that T cells are the only wolves in sheep’s’ clothing, they had better think twice. Recent evidence has shown that a subpopulation of neutrophils (about 5%) in both mouse and human possess the same key elements as T lymphocytes, in expressing the TCR-based immunoreceptors [5]. Similarly it has been shown that synovial neutrophils from patients suffering rheumatoid arthritis can transdifferentiate into dendritic cells and hence express key antigen presenting molecules such as MHC II and CD40, CD80, CD83 and CD86 [6].

1
2 Thus it seems that immune cells, like human beings, like to transgress from their role and change
3
4 their function or phenotype in specific settings. We have also learned that these T cells differ
5
6 from their “conventional” siblings not just in their make-up of cell surface markers, but also in
7
8 their function (Figure 1) since they appear to behave more like innate rather than adaptive
9
10 immune cells.
11
12

13
14
15
16 In this commentary we will be focusing our attention on $CD3^+CD4^-CD8^-$ double negative (DN) T
17
18 cells, and on their pathophysiological functions. In particular, we will provide an overview of
19
20 their origin and development as well as of their role as suppressor (DN regulatory T cells; DN
21
22 Tregs) or effector (DN T cells) of the immune and inflammatory response.
23
24
25

26 27 28 **A very brief overview of T cell development** 29

30
31 Most T cells develop in the thymus, which provides a specialized environment that supports the
32
33 maturation of lymphocyte precursors into functional T-cells [7]. The thymus is seeded by blood-
34
35 borne progenitor cells from the foetal liver or adult bone-marrow which develop along a well-
36
37 characterised programme, dependent on bidirectional signals between the developing thymocytes
38
39 and the thymic epithelial cells (TEC) [8]. During $\alpha\beta$ T-cell development, thymic $CD4^-CD8^-$ DN
40
41 cells give rise to the $CD4^+CD8^+$ double positive (DP) population, which differentiate to mature
42
43 $CD8^+CD4^-$ or $CD8^-CD4^+$ single positive (SP) cells. The DN population can be subdivided by cell
44
45 surface expression of CD25 and CD44. $CD44^+CD25^-$ (DN1) cells differentiate to become
46
47 $CD44^+CD25^+$ (DN2) cells, which then differentiate to become $CD44^-CD25^+$ (DN3). The DN3
48
49 population gives rise to the $CD44^-CD25^-$ (DN4) subset, which undergo a phase of rapid
50
51 proliferation before differentiation into the DP population, in general *via* a cycling immature
52
53 $CD8^+$ intermediate single positive (ISP) cell (Figure 1).
54
55
56
57
58
59
60
61
62
63
64
65

1
2 Maturation from the DP population to the mature SP T-cell populations involves positive
3
4 selection of the $\alpha\beta$ TCR repertoire to ensure appropriate MHC restriction and negative selection
5
6 of potentially self-reactive clones. Many models have been proposed to describe how DP
7
8 thymocytes commit to the CD4 and CD8 lineages, and to explain how positive selection ensures
9
10 that selected CD4 and CD8 SP populations express TCR appropriately restricted by MHC Class
11
12 II and Class I respectively. TCR repertoire selection is dependent on interactions between the
13
14 TCR on the developing thymocyte and its MHC/peptide ligand on TEC [9-11]. Strength and
15
16 duration of TCR signal are thought to broadly determine the DP cell's fate with the strongest
17
18 signals leading to negative selection and apoptosis, (i.e. in the case of TCR recognizing self
19
20 antigens), intermediate signals leading to positive selection, and, weaker signals or lack of TCR
21
22 signaling leading to DP cell death by neglect [12]. For DP thymocytes that are undergoing
23
24 positive selection, TCR signal strength and duration have been proposed to influence CD4 and
25
26 CD8 lineage commitment, with those cells receiving stronger TCR signals tending to be biased
27
28 towards the CD4 fate, and weaker/more transient signals tending to favor differentiation to CD8
29
30 SP T cells [10]. Transcription factors, such as Th-POK, play essential roles in lineage
31
32 commitment, and the ways in which the transcriptional regulation of lineage commitment relate
33
34 to TCR signaling require further study [11]. In addition, other secreted factors from TEC, such
35
36 as the morphogen Sonic hedgehog influence TCR repertoire selection and differentiation from
37
38 DP to SP cell [13, 14].
39
40
41
42
43
44
45
46
47
48
49

50 **Thymic and extrathymic origin of DN T cells**

51
52
53 DN T cells have been proposed to originate in the thymus by escaping negative selection
54
55 followed by migration in the periphery where they expand upon experiencing antigen [15].
56
57
58 Similar to T helper cell lineage commitment, the strength of TCR signalling seems to be an
59
60 important factor for the development of DN T cells. According to this hypothesis, lower intensity
61
62
63
64
65

1
2 TCR signals would lead typically to the generation of mature CD4⁺ and CD8⁺ T cells while high
3
4 TCR strength that borders deletion would promote the survival and escaping of DN T cells from
5
6 the thymus [16]. Using a model of thymocyte reaggregated culture Wang *et al.* demonstrated that
7
8 stimulation of cells with high concentrations of high affinity antigens leads to the conversion of
9
10 DP thymocytes into DN T cells *via* down-modulation of both CD4 and CD8 coreceptors
11
12 expression [17]. It has alternatively been proposed that some DN T cells arise from the pre-TCR⁺
13
14 DN population (late DN3 population). Under this model, strong signal transduction through the
15
16 pre-TCR/CD3 complex of $\gamma\delta$ TCR complex favours differentiation to DN T cell, whereas
17
18 “normal/average” pre-TCR/CD3 complex signal transduction promotes differentiation to DP cell
19
20 and commitment to the $\alpha\beta$ T cell lineage [18].
21
22
23
24
25
26
27
28

29 Several line of evidences also support the theory that DN T cells are generated in the periphery
30
31 rather than in the thymus. Using CD8-deficient mice and sublethally irradiated thymectomized
32
33 mice reconstituted with T cell-depleted bone marrow cells Ford *et al.* demonstrated that
34
35 functional regulatory DN T cells exist in CD8-deficient mice, hence they are not derived from
36
37 antigen-stimulated CD8⁺ T cells. In contrast, a reduction in DN T cell number was observed in
38
39 reconstituted thymectomized mice compared with nonthymectomized recipients. Interestingly,
40
41 DN T cells that develop in thymectomized mice showed an increased suppressing activity
42
43 compared to those that develop in sham-thymectomized mice [19].
44
45
46
47
48
49

50 Mice lacking the expression of Fas/APO-1/CD95 (*lpr*) or Fas-ligand (*gld*) have been widely used
51
52 to investigate the developmental origin of DN T cells. These mice show a peculiar accumulation
53
54 of DN T cells in the lymph nodes and other organs [20, 21] as a result of an impaired induction of
55
56 apoptosis leading to lack of deletion of autoreactive T cells that is seen in normal mice [22]. *In*
57
58 *vivo* antigenic stimulation of TCR transgenic/*lpr* mice resulted in peripheral deletion of T cells
59
60
61
62
63
64
65

1 and in a nine fold increase in the percentage of DN T cells in the lymph nodes of the CD95-
2 deficient but not the CD95-intact mice [23] suggesting a peripheral origin of these cells during
3 immune stimulation. The clinical relevance of these observations has been confirmed in human.
4 Patients suffering from autoimmune lymphoproliferative syndrome (ALPS) have mutations in the
5 Fas apoptotic pathway that are associated with impaired apoptosis after TCR ligation of single
6 positive (CD4 or CD8) activated mature T cells [24]. Interestingly, these patients share similar
7 clinical features with *lpr* and *gld* mice and a selective accumulation of DN T cells in peripheral
8 blood [25-29].

9
10
11
12
13
14
15
16
17
18
19
20
21
22
23 An extra-thymic origin for DN T cells has been also observed in inflammatory conditions. A
24 selective accumulation of DN T cells has been described in the peritoneal cavity of mice infected
25 with the intracellular pathogen *Listeria monocytogenes* and the appearance of these cells was still
26 observed when adult-thymectomized lethally irradiated bone marrow chimeras mice were tested
27 [30].
28
29
30
31
32
33
34
35
36
37

38 **DN T cells as the villains of the immune system**

39
40 Most of what we know about the pathogenic functions of DN T cells derives from the study of
41 mice expressing a transgenic TCR or from autoimmune-prone genetically modified mice. The
42 artificial expression of the transgenic $\alpha\beta$ TCR at the double negative stage of T cell development
43 has been proposed to mimic signals normally conveyed by the $\gamma\delta$ TCR and thus lead to the
44 generation of an artificial subpopulation of T cells with a unique profile of surface markers and
45 cytokine production. One example of these mice is the HY-TCR transgenic mice. HY mice
46 express a transgenic TCR that recognizes the male HY antigen presented by MHC class I
47 molecule. In female mice the HY transgenic T cells are positively selected to the CD8 lineage.
48 Conversely, male TCR transgenic mice show a strong reduction of the DP thymocytes and CD8⁺
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 transgenic T cells [31, 32]. Interestingly, in male mice a relatively small number of DN T cells
2
3
4 escape thymic selection and can be recovered in the periphery or as intraepithelial gut
5
6 lymphocytes [31, 33].
7

8
9
10 Analysis of the gene fingerprint of these cells by microarray demonstrated that, similar to
11
12 $\gamma\delta$ TCR or NK T cells, HY-TCR+ DN T cells showed a “hair-trigger” reactivity that place them in
13
14 an intermediate position between innate and adaptive cells [34, 35]. In fact these cells showed an
15
16 unexpected upregulation of genes associated with the innate immune system and an increased
17
18 production of IL-2 and IFN- γ when compared to female CD8+ T cells. These findings *in vitro*
19
20 have been recently confirmed *in vivo* by our group using a classical model of delayed type
21
22 hypersensitivity (DTH): the λ -carrageenan-induced paw oedema. Male HY mice showed an
23
24 exacerbated inflammatory response accompanied by accumulation of HY-TCR+ cells in the
25
26 inflamed paw tissues. *Ex vivo* analysis of the cytokine profile expressed by activated T cells
27
28 present in the draining lymph nodes of male HY mice showed an increased production of
29
30 classical DTH cytokines such as IL-2, IFN- γ and TNF- α or other “autoimmune” cytokines such
31
32 as IL-17A and IL-22 compared to female HY-TCR+ cells [36].
33
34
35
36
37
38
39
40
41
42
43

44 The proinflammatory phenotype of mature DN T cells has also been observed during their
45
46 development in the thymus. Interestingly, CD44⁺ DN thymocytes have been found to produce
47
48 high levels of IL-2, TNF- α and IFN- γ upon stimulation with PMA/Ionomycin while CD44⁻ DN
49
50 did not produce any of the cytokines mentioned. Similarly, stimulation of $\alpha\beta$ TCR expressing DN
51
52 thymocytes and DN isolated from the spleen with anti-CD3 revealed a similar pattern with IL-4,
53
54 IFN- γ and TNF- α being the main cytokines released upon activation [37].
55
56
57
58
59
60
61
62
63
64
65

1 A growing number of recent studies suggest that DN T cells are not just an artificial subsets of
2 cells occurring in transgenic or genetically modified mice but key players in autoimmunity and
3
4 inflammation in humans. Several studies provided a direct link between DN T cells and the
5
6 development of autoimmune diseases. DN T cells have been found in the blood of myasthenia
7
8 gravis patients with lymphofollicular hyperplasia and have been shown to participate in the
9
10 immunoregulation and increased antibody production [38]. Patients suffering from systemic
11
12 lupus erythematosus (SLE) have a significantly expanded population of DN T cells and these
13
14 cells are the major producers of IL-17 [39], one of the key inflammatory cytokine involved in
15
16 SLE [40, 41]. These results confirmed previous observations from the same group showing that
17
18 DN T cells from MRL/lpr mice produce high levels of IL-17 as the disease progresses. In
19
20 addition, *in vitro* treatment of MRL/lpr lymph node cells with IL-23, a cytokine required for
21
22 sustained Th17 cell differentiation [42], induces the generation of highly pathogenic DN T cells
23
24 causing nephritis following adoptive transfer in lymphocyte deficient Rag1^{-/-} mice [43, 44]. In a
25
26 study conducted on patients suffering from ALPS [45] (also known as Canale-Smith syndrome)
27
28 the authors demonstrated that the percentage of DN T cells increased from 1% (observed in
29
30 physiological conditions) to 40% [46]. These results together with other evidence [26-29] suggest
31
32 that the increased number of DN T cells may represent a sensitive test or biomarker to screen
33
34 patients who should undergo testing for ALPS. In this regard it is interesting to mention that the
35
36 analysis of DN T cell levels has been used to distinguish ALPS from other autoimmune disease
37
38 such as Evans syndrome [27] or sarcoidosis [47].
39
40
41
42
43
44
45
46
47
48
49
50
51
52

53 In line with their ability to produce high levels of IL-17, DN T cells have been reported to play a
54
55 key role in various models of infections. DN T cells are the major responding T cell subset in the
56
57 lung of mice infected with live vaccine strain (LSV) of the intracellular bacterium *F. Tularensis*.
58
59 Temporal analysis of cytokine production in infected lung showed that DN T cells release large
60
61
62
63
64
65

1 amount of IL-17A during the early stages of infection (7 days) while CD4⁺ T cells start to
2 produce this cytokine later (14 days) [48]. Considering that IL-17-deficient mice die at day 8-10
3 post LSV infection [49], it is tempting to suggest that DN T cells rather than CD4⁺ T cells are
4 major contributors to the development of LSV infection. Marodon *et al.* provided further
5 evidence of the importance of DN T cells in infectious diseases. In this study the authors showed
6 that DN T cells of HIV-infected patients represent up to 20% of the cellular viral load in T cells;
7 moreover, successful antiviral therapy was associated with a significant decrease in viral load in
8 DN T cells suggesting that infected DN T cells, like CD4⁺ cells, contribute to viral production
9 and are sensitive to highly active antiretroviral therapy [50].
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25

26 **DN T cells as the saviours of the immune system**

27 T cells with immunoregulatory activity have always attracted the attention of scientists for their
28 potential clinical application in a variety of pathological conditions ranging from autoimmunity
29 to tumour immunology [51, 52]. These cells act as sentinels of the immune system and thus are
30 essential in the maintenance of immune homeostasis and self-tolerance [53].
31
32
33
34
35
36
37
38
39
40

41 The subtypes of T cells with immunoregulatory function has significantly increased in the last
42 few years and we now know that there are Treg other than the originally identified
43 CD4⁺/FoxP3⁺/CD25⁺ T cells. These include NO-Tregs that derive from CD4⁺CD25⁻ T cells and
44 are induced by nitric oxide (NO) [54], Tr1 cells that do not express FoxP3 and release a large
45 amount of IL-10 [55] [56] and TGF- β -producing CD4⁺ Th3 cells [57, 58]. As if this field of
46 research was not already “crowded”, DN T cells have also been added to this list. These cells
47 have been described as the only subtype of immunoregulatory cells able to suppress antigen
48 specific T cell responses [59, 60] as well as a number of other features that make them different
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 from the “conventional” regulatory T cells [61-63]. To avoid confusion with the other DN subset,
2
3 we will refer to these cells as DN Tregs.
4
5

6
7
8
9 DN Tregs have been isolated and identified in mouse, rat and human. One of the first studies
10 conducted in mice described the isolation of natural suppressor DN T cells derived from the
11 spleen of adult mice [64, 65]. A subsequent study on rat DN T cell clones demonstrated that these
12
13 cells did not elicit disease but rather inhibited the development of experimental autoimmune
14
15 encephalomyelitis (EAE) [66]. Studies in human identified a population of DN T cells in a patient
16
17 with immunodeficiency, lymphocytosis, lymphadenopathy, and hepatosplenomegaly. These cells
18
19 showed an impaired proliferative response upon stimulation with mitogens or anti-CD3 plus anti-
20
21 CD28 but were not tested for their immunosuppressive activity [67].
22
23
24
25
26
27
28
29

30
31 As in the case of inflammatory DN T cells, TCR transgenic mice contain a significant number of
32
33 DN Tregs. These cells present a number of features including a similarity with naive CD8⁺ T
34
35 cells that have undergone homeostatic proliferation and conversion into memory-like T cells,
36
37 lower threshold of activation [68] and functional anergy as they hypoproliferate and produce little
38
39 or no IL-2 in response to antigen stimulation [69]. Controversies exist on the possible
40
41 mechanisms by which DN Tregs down-regulate immune responses. According to one study, DN
42
43 Tregs from TCR transgenic mice acquire the antigen from antigen presenting cells *via*
44
45 trogocytosis and they then re-express these newly acquired molecules on their cell surface to
46
47 mediate antigen-specific suppression of activated alloreactive CD8⁺ T cells [62]. Further studies
48
49 have confirmed these findings and have shown that DN Tregs acquire alloantigen *in vivo* *via*
50
51 trogocytosis [70]. Like classical CD4⁺ CD25⁺FoxP3 Treg they require direct cell contact for
52
53 suppression and express a unique combination of cell surface markers that makes them
54
55 distinguishable from any previously described T-cell subsets [62]. According to another study,
56
57
58
59
60
61
62
63
64
65

1 the killing of CD8⁺ T cells by DN Tregs depends on the direct recognition of the antigen on the
2 target cell by the TCR and this enables DN Tregs to respond to a greater variety of self antigens
3 and hence, to regulate the response of a larger repertoire of T cells [15]. Recent studies on human
4 DN Tregs have revealed some discrepancies with their murine counterparts. Human DN Tregs
5 are not believed to eliminate effector T cells by Fas/FasL-mediated apoptosis, modulation of
6 antigen presenting cells or competition for growth factors but to suppress by an active cell
7 contact-dependent mechanism [71].
8
9
10
11
12
13
14
15
16
17
18
19
20

21 Several investigations have shown that DN Tregs play an important role in the development of
22 tolerance after transplantation. DN Tregs have been described to prolong both allo- and xenograft
23 survival compared with untreated controls when adoptively transferred into syngeneic mice.
24 Initial studies in transgenic mice suggested that DN Treg cells can be generated *in vitro* and
25 protect cardiac allograft from rejection when infused into recipients prior to transplantation [72].
26 However, in a subsequent study by Lee *et al.* the authors showed that although single-dose
27 infusion of DN Treg clones from normal mice induces permanent survival of MHC class I-
28 mismatched cardiac allografts, their suppressing activity was lost after *in vitro* culture and clonal
29 expansion [73]. Similarly in another study using rat to mouse cardiac xenotransplant it was found
30 that DN Tregs harvested from the spleens of the recipient mice dose-dependently inhibit the
31 proliferation of syngeneic antidonor T cells [74].
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

50 DN Tregs also play a homeostatic role in autoimmune diseases. Diabetes-prone mice carry fewer
51 DN Tregs and this contributes to the increased susceptibility of these mice to develop the disease.
52 Different mechanisms have been proposed to contribute to this effect. Using a transgenic TCR
53 mouse model Hillhouse and colleagues have shown that *in vivo* antigenic stimulation of
54 transgenic T cells induced a 10 fold increase in the number of DN Tregs in diabetes-prone
55
56
57
58
59
60
61
62
63
64
65

1 NOD.H^{2k} but not in B10.Br control mice. They also showed that IL-10 produced by these cells
2
3 favours their apoptosis and hence helps to regulate their number [75]. In another study by Duncan
4
5 *et al.* the authors proposed a correlation between DN Tregs and the late onset of diabetes in NOD
6
7 mice. Young NOD mice have a high frequency of DN Tregs that declines in adulthood and their
8
9 adoptive transfer in Nod/Scid mice provided a long-lasting protective effect. Consistent with the
10
11 previous study, the authors also demonstrated that the anti-diabetogenic effect of DN Tregs relied
12
13 mainly on the ability to differentiate into IL-10-secreting Tr-1 cells [76].
14
15
16
17
18
19
20

21 **DN T cell “reservoirs” in the body**

22
23 DN T cells seem to preferentially dwell in specific organs or tissues (summarized in Figure 2). In
24
25 MRL-lpr/lpr mice DN T cells were found in close vicinity to Kupffer cells or endothelial cells of
26
27 the hepatic sinusoids. Most interestingly, this liver-specific accumulation of DN T cells is not a
28
29 unique feature of MRL-lpr/lpr but it occurs in several other autoimmune-prone strains including
30
31 C3H/HeJ-gld/gld, BXSB, NOD and NZB/W F1 mice with their proportion increasing as mice
32
33 become old and diseased [77]. The clinical relevance of these findings has been confirmed in a
34
35 study where an analysis of the number of peripheral blood DN T cells in patients after liver
36
37 transplantation was conducted. Patients with proven acute cellular rejection showed a significant
38
39 increase in DN T cells and a significant reduction after pulse corticosteroid therapy [78]. The
40
41 kidney is another favourite hub for DN T cells. However, unlike the liver, the number of DN T
42
43 cells in the kidney decreases during inflammatory conditions such as after ischemia and
44
45 reperfusion [79, 80]. These results are in contrast with the afore mentioned study in which it was
46
47 shown that patients with lupus nephritis have a significant number of DN T cells infiltrating the
48
49 kidney [39].
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 An interesting study investigated the phenotype, distribution, and function of T lymphocytes in
2 the female genital tract of naive, pregnant, or Chlamydia trachomatis-infected C57BL/6 mice.
3
4 Unexpectedly, the authors observed that DN Tregs were the dominant lymphocyte population
5
6 (70-90%) in the genital tract [81]. This might be of clinical importance considering that DN T
7
8 cells have been associated with pathogenesis of Behcet's disease [82], a complex mucosal
9
10 inflammatory disorder with neural and vascular manifestations that includes oral aphtosis and
11
12 genital ulcers [83].
13
14
15
16
17
18
19
20

21 A unique distribution of DN T cells has been described in CD18-deficient mice. Comparative
22
23 analysis of cervical, axillary and inguinal lymph node populations in wild-type and CD18-
24
25 deficient mice showed an expected six fold decrease in the total lymphocyte number in axillary
26
27 and inguinal lymph nodes, together with a fourfold increase in the cellularity of cervical lymph
28
29 nodes, of the latter compared to the former. These cells are massively generated within the lymph
30
31 nodes upon antigen stimulation and display typical features of antigen-experienced cells
32
33 including lower threshold of stimulation, heightened proliferation rate and increased re-
34
35 circulation to non-lymphoid organs [84].
36
37
38
39
40
41
42

43 **Conclusions and perspectives**

44
45 The increasing number of studies on the role and function of DN T cells over the last decade have
46
47 significantly expanded our knowledge of this new subset of immune cells in health and disease.
48
49 Most importantly, we now know that these cells are not just an artefact that occurs in genetically
50
51 modified or transgenic TCR mouse models but that they are also found in a variety of human
52
53 conditions spanning from autoimmune disease to inflammation, infection and cancer [26, 39, 59].
54
55 Table 1 and 2 summarize the main functions of pathological DN T cells and DN Tregs described
56
57 in this commentary.
58
59
60
61
62
63
64
65

1 One of the most intriguing aspects of the biology of DN T cells is their dual identity i.e. their
2 pathogenic or immunosuppressive phenotype. In this context, a number of questions need to be
3
4 addressed. For instance, how do DN T cells decide to be villains or saviours of the immune
5
6 system? Do DN T cells and DN Tregs derive from the same progenitor cell? And most
7
8 importantly, can DN Tregs convert into inflammatory DN T cells? This very last aspect is of
9
10 great importance before we even begin considering to use DN Tregs in cell immunotherapies.
11
12
13
14
15
16
17
18

19 A great deal of attention has been recently given to the concept of T cell plasticity. According to
20
21 this theory, Th effector cells can readily switch from one phenotype to another especially when
22
23 adoptively transferred *in vivo*. Thus, Th17 cells can convert in Th1 cells, classical FoxP3⁺ Treg
24
25 can become Th17 and Th2 cells can change into an IL-9 producing Th9 cells [85-87]. Whether or
26
27 not inflammatory DN T cells can acquire an immunosuppressive phenotype and *vice versa* has
28
29 not been explored yet and it would certainly be very interesting to investigate. Clearly, a number
30
31 of factors contribute to their phenotype and more investigations are needed to fully appreciate the
32
33 intrinsic and extrinsic factors influencing their fate. In any case, several observations indicate
34
35 that, like for Th effector cells, the microenvironment plays a key role in determining DN T cell
36
37 functions and/or expansion.
38
39
40
41
42
43
44

45 Beside these considerations, we think that there are important aspects other than their origin or
46
47 development that have not been fully explored. If one wants to bring DN T cells under the
48
49 spotlight of “translational science”, there are several gaps that need to be filled in. First of all, the
50
51 identification of specific markers that allow distinction between inflammatory DN T cells from
52
53 DN Tregs would be of great clinical and therapeutical importance. A first attempt has been
54
55 carried out by Lee and colleagues by comparing gene expression differences between functional
56
57 DN Treg cells and nonfunctional mutants [73] but clearly more extensive proteomic analyses are
58
59
60
61
62
63
64
65

1 needed. In other studies it has been found that *lpr* DN lymph node cells are homogenous for
2 expression of high level of CD44, B220 (the isoform of CD45 found on B cells) [88, 89], CXCR5
3
4 [90] and lack the expression of CD2 [91] and IL-2 receptor[92].
5
6
7
8
9

10 The availability of further specific markers will facilitate the isolation and analysis of these cells
11 and, at the same time, might provide novel diagnostic tools that can be used to predict disease
12 progression and possibly remission. Further insights into the mechanisms of activation of DN T
13 cells could be used to investigate at the biochemical level what makes these cells so hyperreactive
14 and pathogenic. This information could be used for the design of specific drugs able to limit
15 inflammatory DN T activation or, possibly, favour DN Treg development and/or expansion.
16
17
18
19
20
21
22
23
24
25

26 We recognize that these are not simple and straightforward processes, especially if one aims at
27 ultimately targeting human DN T cells. However, there are small steps that can (and should) be
28 taken up by pharmacologists interested in immune and inflammatory diseases. In our view, the
29 availability of relatively simple experimental systems to study DN T cells or DN Tregs would
30 provide a significant boost in translational research in this area. For instance, in our recent study
31 we have used the HY-TCR transgenic mice to assess the contribution of DN T cells to the early
32 and late stage of the inflammatory reaction [36]. The high frequency of DN T cells in male mice
33 makes these animals an ideal system to test novel therapeutic strategies targeting these cells.
34 Similarly, given the high number of DN Treg in young NOD mice [76], this system could be an
35 ideal platform for the testing of novel immunosuppressive therapies.
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54

55 Along these lines, it is somehow surprising that very little attention has been given to the possible
56 effects of commonly used anti-inflammatory and immunosuppressive drugs on DN T cells or DN
57 Tregs. Evidence that popular pharmacological therapies might modulate CD4 and CD8
58
59
60
61
62
63
64
65

1 expression in T cells were reported more than 20 years ago [93-95] and thus the possibility that
2 these drugs might act on DN T cells and DN Treg development and/or activation is not far
3 fetched. In one interesting recent studies by Okazaki et al. the authors showed that FTY720, a
4 novel immunosuppressant that modulates sphingosine 1-phosphate receptor, induced apoptosis in
5 more than 70% of DN T cells from the spleen of MRL/lpr mice *in vitro* and *in vivo* [96].
6
7
8
9
10
11
12
13
14
15

16 To conclude, we hope that this commentary will help the readers of Biochemical Pharmacology
17 to appreciate the importance of DN T cells as therapeutic targets since we think that this is a
18 neglected area of research that needs much attention and which has great potential for
19 pharmacological intervention. We also hope that this overview will invite investigators to look
20 back at their cytofluorimetric or immunohistochemistry data with new eyes that are not
21 obfuscated by the analysis of solely CD4 or CD8 single positive T cells.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

REFERENCES

- [1] Medzhitov R. Origin and physiological roles of inflammation. *Nature* 2008;454:428-35.
- [2] Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol* 2007;7:678-89.
- [3] Joly E, Hudrisier D. What is trogocytosis and what is its purpose? *Nat Immunol* 2003;4:815.
- [4] Hudrisier D, Aucher A, Puaux AL, Bordier C, Joly E. Capture of target cell membrane components via trogocytosis is triggered by a selected set of surface molecules on T or B cells. *J Immunol* 2007;178:3637-47.
- [5] Puellmann K, Kaminski WE, Vogel M, Nebe CT, Schroeder J, Wolf H, et al. A variable immunoreceptor in a subpopulation of human neutrophils. *Proc Natl Acad Sci U S A* 2006;103:14441-6.
- [6] Iking-Konert C, Ostendorf B, Sander O, Jost M, Wagner C, Joosten L, et al. Transdifferentiation of polymorphonuclear neutrophils to dendritic-like cells at the site of inflammation in rheumatoid arthritis: evidence for activation by T cells. *Ann Rheum Dis* 2005;64:1436-42.
- [7] Ciofani M, Zuniga-Pflucker JC. The thymus as an inductive site for T lymphopoiesis. *Annu Rev Cell Dev Biol* 2007;23:463-93.
- [8] Petrie HT, Zuniga-Pflucker JC. Zoned out: functional mapping of stromal signaling microenvironments in the thymus. *Annu Rev Immunol* 2007;25:649-79.
- [9] Kappes DJ, He X. CD4-CD8 lineage commitment: an inside view. *Nat Immunol* 2005;6:761-6.
- [10] Bommhardt U, Basson MA, Krummrei U, Zamoyska R. Activation of the extracellular signal-related kinase/mitogen-activated protein kinase pathway discriminates CD4 versus CD8 lineage commitment in the thymus. *J Immunol* 1999;163:715-22.
- [11] He X, Dave VP, Zhang Y, Hua X, Nicolas E, Xu W, et al. The zinc finger transcription factor Th-POK regulates CD4 versus CD8 T-cell lineage commitment. *Nature* 2005;433:826-33.
- [12] von Boehmer H, Kisielow P. Negative selection of the T-cell repertoire: where and when does it occur? *Immunol Rev* 2006;209:284-9.
- [13] Hager-Theodorides AL, Furmanski AL, Ross SE, Outram SV, Rowbotham NJ, Crompton T. The Gli3 transcription factor expressed in the thymus stroma controls thymocyte negative selection via Hedgehog-dependent and -independent mechanisms. *J Immunol* 2009;183:3023-32.
- [14] Rowbotham NJ, Hager-Theodorides AL, Cebecauer M, Shah DK, Drakopoulou E, Dyson J, et al. Activation of the Hedgehog signaling pathway in T-lineage cells inhibits TCR repertoire selection in the thymus and peripheral T-cell activation. *Blood* 2007;109:3757-66.
- [15] Priatel JJ, Utting O, Teh HS. TCR/self-antigen interactions drive double-negative T cell peripheral expansion and differentiation into suppressor cells. *J Immunol* 2001;167:6188-94.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [16] Mixer PF, Russell JQ, Morrissette GJ, Charland C, Aleman-Hoey D, Budd RC. A model for the origin of TCR-alpha/beta+ CD4-CD8- B220+ cells based on high affinity TCR signals. *J Immunol* 1999;162:5747-56.
- [17] Wang R, Wang-Zhu Y, Grey H. Interactions between double positive thymocytes and high affinity ligands presented by cortical epithelial cells generate double negative thymocytes with T cell regulatory activity. *Proc Natl Acad Sci U S A* 2002;99:2181-6.
- [18] Hayes SM, Li L, Love PE. TCR signal strength influences alpha/beta/gammadelta lineage fate. *Immunity* 2005;22:583-93.
- [19] Ford MS, Zhang ZX, Chen W, Zhang L. Double-negative T regulatory cells can develop outside the thymus and do not mature from CD8+ T cell precursors. *J Immunol* 2006;177:2803-9.
- [20] Shirai T, Abe M, Yagita H, Okumura K, Morse HC, 3rd, Davidson WF. The expanded populations of CD4-CD8- T cell receptor alpha/beta+ T cells associated with the *lpr* and *gld* mutations are CD2+. *J Immunol* 1990;144:3756-61.
- [21] Watanabe D, Suda T, Hashimoto H, Nagata S. Constitutive activation of the Fas ligand gene in mouse lymphoproliferative disorders. *EMBO J* 1995;14:12-8.
- [22] Mohamood AS, Bargatze D, Xiao Z, Jie C, Yagita H, Ruben D, et al. Fas-mediated apoptosis regulates the composition of peripheral alpha/beta T cell repertoire by constitutively purging out double negative T cells. *PLoS One* 2008;3:e3465.
- [23] Mehal WZ, Crispe IN. TCR ligation on CD8+ T cells creates double-negative cells in vivo. *J Immunol* 1998;161:1686-93.
- [24] Fisher GH, Rosenberg FJ, Straus SE, Dale JK, Middleton LA, Lin AY, et al. Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell* 1995;81:935-46.
- [25] Fleisher TA. The autoimmune lymphoproliferative syndrome: an experiment of nature involving lymphocyte apoptosis. *Immunol Res* 2008;40:87-92.
- [26] Poppema S, Maggio E, van den Berg A. Development of lymphoma in Autoimmune Lymphoproliferative Syndrome (ALPS) and its relationship to Fas gene mutations. *Leuk Lymphoma* 2004;45:423-31.
- [27] Teachey DT, Manno CS, Axsom KM, Andrews T, Choi JK, Greenbaum BH, et al. Unmasking Evans syndrome: T-cell phenotype and apoptotic response reveal autoimmune lymphoproliferative syndrome (ALPS). *Blood* 2005;105:2443-8.
- [28] Marlies A, Udo G, Juergen B, Bernd S, Herrmann M, Haas JP. The expanded double negative T cell populations of a patient with ALPS are not clonally related to CD4+ or to CD8+ T cells. *Autoimmunity* 2007;40:299-301.
- [29] Cerutti E, Campagnoli MF, Ferretti M, Garelli E, Crescenzo N, Rosolen A, et al. Co-inherited mutations of Fas and caspase-10 in development of the autoimmune lymphoproliferative syndrome. *BMC Immunol* 2007;8:28.
- [30] Kadena T, Matsuzaki G, Fujise S, Kishihara K, Takimoto H, Sasaki M, et al. TCR alpha/beta+ CD4- CD8- T cells differentiate extrathymically in an lck-independent manner and participate in early response against *Listeria monocytogenes* infection through interferon-gamma production. *Immunology* 1997;91:511-9.
- [31] von Boehmer H, Kirberg J, Rocha B. An unusual lineage of alpha/beta T cells that contains autoreactive cells. *J Exp Med* 1991;174:1001-8.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [32] Kisielow P, Bluthmann H, Staerz UD, Steinmetz M, von Boehmer H. Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD4+8+ thymocytes. *Nature* 1988;333:742-6.
- [33] Egawa T, Kreslavsky T, Littman DR, von Boehmer H. Lineage diversion of T cell receptor transgenic thymocytes revealed by lineage fate mapping. *PLoS One* 2008;3:e1512.
- [34] Yamagata T, Benoist C, Mathis D. A shared gene-expression signature in innate-like lymphocytes. *Immunol Rev* 2006;210:52-66.
- [35] Yamagata T, Mathis D, Benoist C. Self-reactivity in thymic double-positive cells commits cells to a CD8 alpha alpha lineage with characteristics of innate immune cells. *Nat Immunol* 2004;5:597-605.
- [36] Maione F, Paschalidis N, Iqbal AJ, Crompton T, Perretti M, D'Acquisto F. Analysis of the inflammatory response in HY-TCR transgenic mice highlights the pathogenic potential of CD4- CD8- T cells. *Autoimmunity* 43:672-81.
- [37] Zlotnik A, Godfrey DI, Fischer M, Suda T. Cytokine production by mature and immature CD4-CD8- T cells. Alpha beta-T cell receptor+ CD4-CD8- T cells produce IL-4. *J Immunol* 1992;149:1211-5.
- [38] Reinhardt C, Melms A. Normalization of elevated CD4-/CD8- (double-negative) T cells after thymectomy parallels clinical remission in myasthenia gravis associated with thymic hyperplasia but not thymoma. *Ann Neurol* 2000;48:603-8.
- [39] Crispin JC, Oukka M, Bayliss G, Cohen RA, Van Beek CA, Stillman IE, et al. Expanded double negative T cells in patients with systemic lupus erythematosus produce IL-17 and infiltrate the kidneys. *J Immunol* 2008;181:8761-6.
- [40] Crispin JC, Tsokos GC. IL-17 in systemic lupus erythematosus. *J Biomed Biotechnol* 2010:943254.
- [41] Nalbandian A, Crispin JC, Tsokos GC. Interleukin-17 and systemic lupus erythematosus: current concepts. *Clin Exp Immunol* 2009;157:209-15.
- [42] Tato CM, Cua DJ. Reconciling id, ego, and superego within interleukin-23. *Immunol Rev* 2008;226:103-11.
- [43] Kyttaris VC, Zhang Z, Kuchroo VK, Oukka M, Tsokos GC. Cutting edge: IL-23 receptor deficiency prevents the development of lupus nephritis in C57BL/6-lpr/lpr mice. *J Immunol* 184:4605-9.
- [44] Zhang Z, Kyttaris VC, Tsokos GC. The role of IL-23/IL-17 axis in lupus nephritis. *J Immunol* 2009;183:3160-9.
- [45] Sneller MC, Straus SE, Jaffe ES, Jaffe JS, Fleisher TA, Stetler-Stevenson M, et al. A novel lymphoproliferative/autoimmune syndrome resembling murine lpr/gld disease. *J Clin Invest* 1992;90:334-41.
- [46] Bleesing JJ, Brown MR, Dale JK, Straus SE, Lenardo MJ, Puck JM, et al. TcR-alpha/beta(+) CD4(-)CD8(-) T cells in humans with the autoimmune lymphoproliferative syndrome express a novel CD45 isoform that is analogous to murine B220 and represents a marker of altered O-glycan biosynthesis. *Clin Immunol* 2001;100:314-24.
- [47] Mullauer L, Emhofer J, Wohlfart S, Pichlhofer B, Stary S, Ebetsberger G, et al. Autoimmune lymphoproliferative syndrome (ALPS) caused by Fas (CD95) mutation mimicking sarcoidosis. *Am J Surg Pathol* 2008;32:329-34.
- [48] Cowley SC, Meierovics AI, Frelinger JA, Iwakura Y, Elkins KL. Lung CD4-CD8-double-negative T cells are prominent producers of IL-17A and IFN-gamma

- 1 during primary respiratory murine infection with *Francisella tularensis* live
2 vaccine strain. *J Immunol* 184:5791-801.
- 3
- 4 [49] Lin Y, Ritchea S, Logar A, Slight S, Messmer M, Rangel-Moreno J, et al.
5 Interleukin-17 is required for T helper 1 cell immunity and host resistance to
6 the intracellular pathogen *Francisella tularensis*. *Immunity* 2009;31:799-810.
- 7
- 8 [50] Marodon G, Warren D, Filomio MC, Posnett DN. Productive infection of double-
9 negative T cells with HIV in vivo. *Proc Natl Acad Sci U S A* 1999;96:11958-63.
- 10
- 11 [51] Gajewski TF, Chesney J, Curriel TJ. Emerging strategies in regulatory T-cell
12 immunotherapies. *Clin Adv Hematol Oncol* 2009;7:1-10; quiz 1-2.
- 13
- 14 [52] Verbsky JW. Therapeutic use of T regulatory cells. *Curr Opin Rheumatol*
15 2007;19:252-8.
- 16
- 17 [53] Valencia X, Lipsky PE. CD4+CD25+FoxP3+ regulatory T cells in autoimmune
18 diseases. *Nat Clin Pract Rheumatol* 2007;3:619-26.
- 19
- 20 [54] Niedbala W, Cai B, Liu H, Pitman N, Chang L, Liew FY. Nitric oxide induces
21 CD4+CD25+ Foxp3 regulatory T cells from CD4+CD25 T cells via p53, IL-2, and
22 OX40. *Proc Natl Acad Sci U S A* 2007;104:15478-83.
- 23
- 24 [55] O'Garra A, Vieira PL, Vieira P, Goldfeld AE. IL-10-producing and naturally
25 occurring CD4+ Tregs: limiting collateral damage. *J Clin Invest* 2004;114:1372-
26 8.
- 27
- 28 [56] Mills KH, McGuirk P. Antigen-specific regulatory T cells--their induction and
29 role in infection. *Semin Immunol* 2004;16:107-17.
- 30
- 31 [57] Horwitz DA, Zheng SG, Gray JD, Wang JH, Ohtsuka K, Yamagiwa S. Regulatory T
32 cells generated ex vivo as an approach for the therapy of autoimmune disease.
33 *Semin Immunol* 2004;16:135-43.
- 34
- 35 [58] Zheng SG, Gray JD, Ohtsuka K, Yamagiwa S, Horwitz DA. Generation ex vivo of
36 TGF-beta-producing regulatory T cells from CD4+CD25- precursors. *J Immunol*
37 2002;169:4183-9.
- 38
- 39 [59] Thomson CW, Lee BP, Zhang L. Double-negative regulatory T cells: non-
40 conventional regulators. *Immunol Res* 2006;35:163-78.
- 41
- 42 [60] Zhang ZX, Young K, Zhang L. CD3+CD4-CD8- alphabeta-TCR+ T cell as immune
43 regulatory cell. *J Mol Med* 2001;79:419-27.
- 44
- 45 [61] Fischer K, Voelkl S, Heymann J, Przybylski GK, Mondal K, Laumer M, et al.
46 Isolation and characterization of human antigen-specific TCR alpha beta+
47 CD4(-)CD8- double-negative regulatory T cells. *Blood* 2005;105:2828-35.
- 48
- 49 [62] Zhang ZX, Yang L, Young KJ, DuTemple B, Zhang L. Identification of a previously
50 unknown antigen-specific regulatory T cell and its mechanism of suppression.
51 *Nat Med* 2000;6:782-9.
- 52
- 53 [63] Young KJ, Yang L, Phillips MJ, Zhang L. Donor-lymphocyte infusion induces
54 transplantation tolerance by activating systemic and graft-infiltrating double-
55 negative regulatory T cells. *Blood* 2002;100:3408-14.
- 56
- 57 [64] Schwadron RB, Palathumpat V, Strober S. Natural suppressor cells derived
58 from adult spleen and thymus. *Transplantation* 1989;48:107-10.
- 59
- 60 [65] Strober S, Cheng L, Zeng D, Palathumpat R, Dejbakhsh-Jones S, Huie P, et al.
61 Double negative (CD4-CD8- alpha beta+) T cells which promote tolerance
62 induction and regulate autoimmunity. *Immunol Rev* 1996;149:217-30.
- 63
- 64 [66] Lider O, Miller A, Miron S, Hershkovich R, Weiner HL, Zhang XM, et al.
65 Nonencephalitogenic CD4-CD8- V alpha 2V beta 8.2+ anti-myelin basic protein
rat T lymphocytes inhibit disease induction. *J Immunol* 1991;147:1208-13.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [67] Illum N, Ralfkiaer E, Pallesen G, Geisler C. Phenotypical and functional characterization of double-negative (CD4-CD8-) alpha beta T-cell receptor positive cells from an immunodeficient patient. *Scand J Immunol* 1991;34:635-45.
- [68] Utting O, Teh SJ, Teh HS. A population of in vivo anergized T cells with a lower activation threshold for the induction of CD25 exhibit differential requirements in mobilization of intracellular calcium and mitogen-activated protein kinase activation. *J Immunol* 2000;164:2881-9.
- [69] Caveno J, Zhang Y, Motyka B, Teh SJ, Teh HS. Functional similarity and differences between selection-independent CD4-CD8- alphabeta T cells and positively selected CD8 T cells expressing the same TCR and the induction of anergy in CD4-CD8- alphabeta T cells in antigen-expressing mice. *J Immunol* 1999;163:1222-9.
- [70] Ford McIntyre MS, Young KJ, Gao J, Joe B, Zhang L. Cutting edge: in vivo trogocytosis as a mechanism of double negative regulatory T cell-mediated antigen-specific suppression. *J Immunol* 2008;181:2271-5.
- [71] Voelkl S, Gary R, Mackensen A. Characterization of the immunoregulatory function of human TCR-alphabeta+ CD4- CD8- double-negative T cells. *Eur J Immunol* 41:739-48.
- [72] Chen W, Ford MS, Young KJ, Zhang L. Infusion of in vitro-generated DN T regulatory cells induces permanent cardiac allograft survival in mice. *Transplant Proc* 2003;35:2479-80.
- [73] Lee BP, Mansfield E, Hsieh SC, Hernandez-Boussard T, Chen W, Thomson CW, et al. Expression profiling of murine double-negative regulatory T cells suggest mechanisms for prolonged cardiac allograft survival. *J Immunol* 2005;174:4535-44.
- [74] Chen W, Ford MS, Young KJ, Cybulsky MI, Zhang L. Role of double-negative regulatory T cells in long-term cardiac xenograft survival. *J Immunol* 2003;170:1846-53.
- [75] Hillhouse EE, Beauchamp C, Chabot-Roy G, Dugas V, Lesage S. Interleukin-10 limits the expansion of immunoregulatory CD4-CD8- T cells in autoimmune-prone non-obese diabetic mice. *Immunol Cell Biol* 88:771-80.
- [76] Duncan B, Nazarov-Stoica C, Surls J, Kehl M, Bona C, Casares S, et al. Double negative (CD3+ 4- 8-) TCR alphabeta splenic cells from young NOD mice provide long-lasting protection against type 1 diabetes. *PLoS One* 5:e11427.
- [77] Masuda T, Ohteki T, Abo T, Seki S, Nose S, Nagura H, et al. Expansion of the population of double negative CD4-8- T alpha beta-cells in the liver is a common feature of autoimmune mice. *J Immunol* 1991;147:2907-12.
- [78] Crosbie OM, Costello PJ, O'Farrelly C, Hegarty JE. Changes in peripheral blood double-negative T-lymphocyte (CD3+ CD4- CD8-) populations associated with acute cellular rejection after liver transplantation. *Liver Transpl Surg* 1998;4:141-5.
- [79] Ascon DB, Ascon M, Satpute S, Lopez-Briones S, Racusen L, Colvin RB, et al. Normal mouse kidneys contain activated and CD3+CD4- CD8- double-negative T lymphocytes with a distinct TCR repertoire. *J Leukoc Biol* 2008;84:1400-9.
- [80] Ascon DB, Lopez-Briones S, Liu M, Ascon M, Savransky V, Colvin RB, et al. Phenotypic and functional characterization of kidney-infiltrating lymphocytes in renal ischemia reperfusion injury. *J Immunol* 2006;177:3380-7.

- 1
2 [81] Johansson M, Lycke N. A unique population of extrathymically derived alpha
3 beta TCR+CD4-CD8- T cells with regulatory functions dominates the mouse
4 female genital tract. *J Immunol* 2003;170:1659-66.
- 5 [82] Ling E, Shubinsky G, Press J. Increased proportion of CD3+CD4-CD8- double-
6 negative T cells in peripheral blood of children with Behcet's disease.
7 *Autoimmun Rev* 2007;6:237-40.
- 8 [83] Alpsy E. Behcet's disease: treatment of mucocutaneous lesions. *Clin Exp*
9 *Rheumatol* 2005;23:532-9.
- 10 [84] Oreshkova T, Wang H, Seier AM, Sindrilaru A, Varga G, Grabbe S, et al. Beta(2)
11 integrin deficiency yields unconventional double-negative T cells distinct from
12 mature classical natural killer T cells in mice. *Immunology* 2009;128:271-86.
- 13 [85] Bluestone JA, Mackay CR, O'Shea JJ, Stockinger B. The functional plasticity of T
14 cell subsets. *Nat Rev Immunol* 2009;9:811-6.
- 15 [86] Zhou L, Chong MM, Littman DR. Plasticity of CD4+ T cell lineage differentiation.
16 *Immunity* 2009;30:646-55.
- 17 [87] Rowell E, Wilson CB. Programming perpetual T helper cell plasticity. *Immunity*
18 2009;30:7-9.
- 19 [88] Budd RC, Schreyer M, Miescher GC, MacDonald HR. T cell lineages in the
20 thymus of *lpr/lpr* mice. Evidence for parallel pathways of normal and
21 abnormal T cell development. *J Immunol* 1987;139:2200-10.
- 22 [89] Cohen PL, Eisenberg RA. *lpr* and *gld*: single gene models of systemic
23 autoimmunity and lymphoproliferative disease. *Annu Rev Immunol*
24 1991;9:243-69.
- 25 [90] Lee BP, Chen W, Shi H, Der SD, Forster R, Zhang L. CXCR5/CXCL13 interaction is
26 important for double-negative regulatory T cell homing to cardiac allografts. *J*
27 *Immunol* 2006;176:5276-83.
- 28 [91] Budd RC, Russell JQ, van Houten N, Cooper SM, Yagita H, Wolfe J. CD2
29 expression correlates with proliferative capacity of alpha beta + or gamma
30 delta + CD4-CD8- T cells in *lpr* mice. *J Immunol* 1992;148:1055-64.
- 31 [92] Budd RC, MacDonald HR, Lowenthal JW, Davignon JL, Izui S, Cerottini JC.
32 Growth and differentiation in vitro of the accumulating *Lyt-2-/L3T4-* subset in
33 *lpr* mice. *J Immunol* 1985;135:3704-11.
- 34 [93] Ramirez F, McKnight AJ, Silva A, Mason D. Glucocorticoids induce the
35 expression of CD8 alpha chains on concanavalin A-activated rat CD4+ T cells:
36 induction is inhibited by rat recombinant interleukin 4. *J Exp Med*
37 1992;176:1551-9.
- 38 [94] Haczku AF, Kay AB, Corrigan CJ. Inhibition of re-expression of surface CD4, but
39 not CD8, on activated human T-lymphocytes by the immunosuppressive drugs
40 dexamethasone and cyclosporine A: correlation with inhibition of
41 proliferation. *Int J Immunopharmacol* 1996;18:45-51.
- 42 [95] Danel-Moore L, Kawa S, Kalmaz GD, Bessman D, Thompson EB. Induction of
43 CD8 antigen and suppressor activity by glucocorticoids in a CEM human
44 leukemic cell clone. *Leuk Res* 1993;17:501-6.
- 45 [96] Okazaki H, Hirata D, Kamimura T, Sato H, Iwamoto M, Yoshio T, et al. Effects of
46 FTY720 in *MRL-lpr/lpr* mice: therapeutic potential in systemic lupus
47 erythematosus. *J Rheumatol* 2002;29:707-16.
- 48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4 Figure 1. Schematic representation of T cell development in the thymus. Progenitor (PG) cells
5
6 from the bone marrow colonize the thymus where they commit to natural killer cells (NK) or T
7
8 cell lineage. Here they undergo progressive differentiation from double-negative (DN) to double-
9
10 positive (DP) to single-positive CD4 or CD8 thymocytes. The immature DN thymocytes can be
11
12 further divided into DN1 to DN4 based on their expression of CD44 and CD25. The $\gamma\delta$ lineage
13
14 diverge from the $\alpha\beta$ at the DN2/DN3 stage. Progression from DP stage to SP can occurs through
15
16 an immature CD8+ single positive (ISP) cell intermediate
17
18
19
20
21
22
23

24 Figure 2. Schematic representation of favourite tissue localization of DN T cells in murine and
25
26 human tissues and their relative frequency in health and disease conditions.
27
28
29
30

31 Table 1. Summary of the main functions of human and murine pathogenic DN T cells.
32
33
34
35

36 Table 2. Summary of the main functions of human and murine DN Tregs cells.
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Table 1

Mouse models or human disease	Phenotype	Reference
HY-TCR transgenic mice	“Hair-trigger” reactivity Production of high levels of IL-2 and IFN- γ	34,35
	Exacerbated inflammatory response <i>in vivo</i> Production of high levels of IL-2, IFN- γ , TNF- α , IL-17A and IL-22	36
MRL/lpr mice	Expansion with disease progression Production of high levels of IL-17	39
	Highly pathogenic following adoptive transfer Expansion following treatment with IL-23	43, 44
Mice infected with F. Tularensis	Release large amount of IL-17A during the early stage of infection	48
Myasthenia gravis patients	Immunoregulatory activity Number correlates with disease activity	38
HIV-infected patients	Represent up to 20% of the cellular viral load in T cells Successful antiviral therapy was associated with a significant decrease in viral load in DN T cells	50
Autoimmune lymphoproliferative syndrome	Increased number/selective expansion	25, 26, 27, 28, 29

Table 2

Mouse models or human disease	Phenotype	Reference
Lewis rats immunized with guinea-pig myelin basic protein	Inhibit the development of experimental autoimmune encephalomyelitis	66
H-2 ^b 2C TCR-transgenic mice	Lower threshold of activation Functional anergy Hypoproliferation and little or no production of IL-2 in response to antigen stimulation	68 69
2C _{F1} (H-2 ^{b/d} , L ^d , anti-L ^d 1B2-TCR+) transgenic mice	Antigen-specific suppression of activated alloreactive CD8+ T cells <i>via</i> trogocytosis	62, 70
Anti-L(d) transgenic TCR+ (2C x dm2)F1 mice	Protect cardiac allograft from rejection	72
Heart grafts from Lewis rats heterotopically transplanted into B6 recipients mice	Dose-dependently inhibit the proliferation of syngeneic antidonor T cells	74
Diabetes-prone NOD.H2k mice	Increase in number after antigenic stimulation <i>in vivo</i>	75
Diabetes-prone NOD mice	Adoptive transfer in Nod/Scid mice provided a long-lasting protective effect	76
Human peripheral blood T cells	Suppress by an active cell contact-dependent mechanism	71

Figure 1

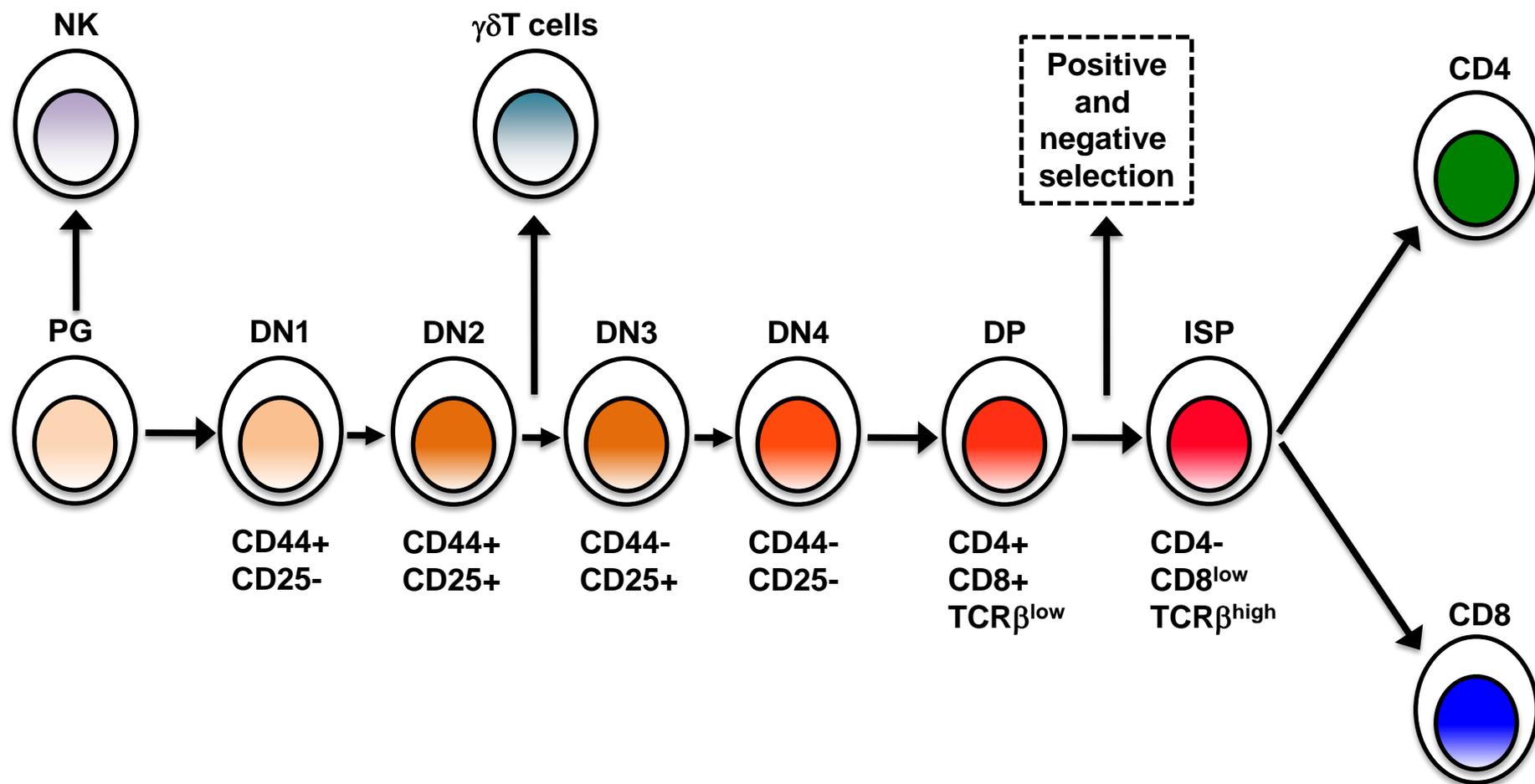
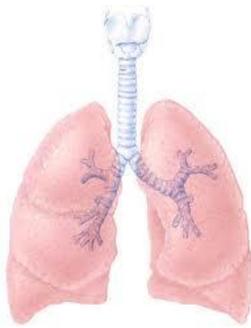
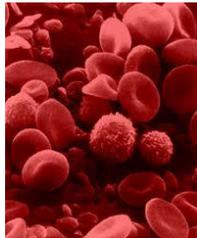


Figure 2

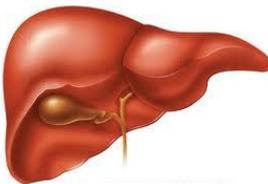
Double negative T cell reservoirs in human body



Lung
↑ Number after infection



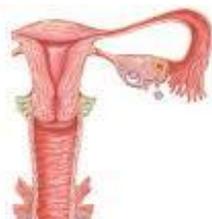
Peripheral blood
1-2% in physiological conditions
Up to 40% in autoimmune conditions



Liver
↑ Number after transplantation



Kidney
↓ Number after ischemia/reperfusion



Genital tract
Up to 70-90% of the total lymphocyte population

The double nature of CD3⁺ CD4⁻CD8⁻ T cells

PATHOGENIC

“Hair trigger” reactivity

Release large amount
of IL-17, IL-2 and IFN- γ

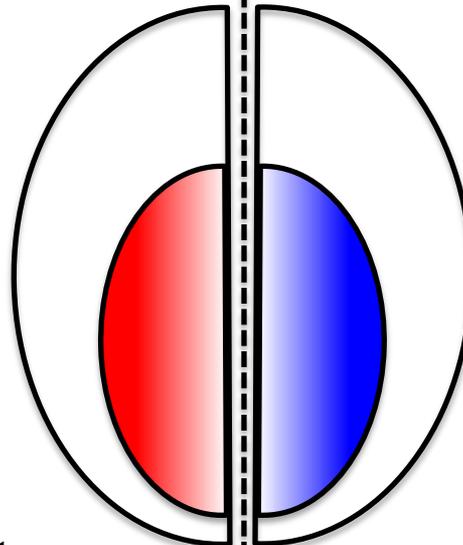
Cellular HIV reservoirs

Increased number
during:

myasthenia gravis

systemic lupus erythematosus

autoimmune lymphoproliferative
syndrome



REGULATORY

Antigen-specific suppression of
alloreactive CD8⁺ T cells

Suppress by an active
cell contact-dependent
mechanism

Protect cardiac
allograft from rejection

Provide a long-lasting
protective effect
in diabetes-prone
NOD mice

Inhibit the development of
experimental
autoimmune encephalomyelitis