



Review

Development and Selection of T Cells: How Many Subsets? How Many Rules?

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Abstract. Since the discovery indicating that thymus-derived lymphocytes (T cells) can be divided into two subpopulations: CD8⁺ (killer) and CD4⁺ (helper) cells, subsequent studies revealed a bewildering heterogeneity of T cells. In the present review an attempt is made to present the current picture of T cell heterogeneity, introduce some order into the nomenclature, and summarize the rules behind the development and selection of different currently recognized T cell subsets.

Key words: thymus; T cell development; T cell heterogeneity; positive selection; negative selection; T cell subsets.

Introduction

In the past, before 1983, T cells were defined as thymus-derived lymphocytes which recognize antigen in the context of the major histocompatibility complex (MHC) molecules and are responsible for cell-mediated immune reactions. Since 1983, after the discovery of T cell antigen receptors (TCR) this definition has gradually become obsolete because it turned out that none of the above-mentioned features characterize all cells bearing TCR molecules.

At present, T cells are defined as lymphocytes equipped with heterodimeric cell surface receptors which are encoded in four somatically rearranging TCR loci: α and β or γ and δ . Depending on the type of expressed TCR (i.e. whether it is encoded in α and β or in γ and δ loci), one can distinguish $\alpha\beta$ and $\gamma\delta$ T cells.

Discussions of T cell heterogeneity are often confounded by interchangeable use of such terms as “sub-

set”, “class” and “subpopulation”. This is so because our knowledge of developmental history of different groups of T cells is often incomplete. However, in spite of this limitation, I would like to propose the following definitions of the above-mentioned terms in order to facilitate the present and future discussion of T cell heterogeneity.

A “T cell subset” refers to a group of terminally differentiated T cells with different developmental history, distinguishable by specific, genetically encoded marker(s) expressed in response to developmental cues, i.e. without intentional priming. Thus, according to this definition, naive CD4⁺8⁻ and CD4⁻8⁺ T cells represent T cell subsets, whereas naive, effector and memory T cells as well as T helper (Th)1 and Th2 T cells do not represent T cell subsets but different activation or maturation states of the same T cell subset. The above definition of a subset resembles the definition of a cell lineage, but a difference is that the term “subset” ap-

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plies to terminally differentiated T cells, whereas “cell lineage” is a broader term referring to all developmental stages of a subset.

A “T cell class” refers to a group of T cell subsets sharing a common characteristic and distinguished from another group of subsets (class) by a different developmental history, e.g. $\alpha\beta$ T cells and $\gamma\delta$ T cells.

A “T cell subpopulation” (subgroup) is a developmentally neutral term and applies to a group of cells distinguished by any criteria, for example size, buoyant density, etc., and even by the genetically encoded marker if it is unknown that it distinguishes a class or a subset or it is known that it does not distinguish a class or a subset.

Discovery of the Major T Cell Subsets and Classes

The chronology of the discovery of major T cell subpopulations is shown in Table 1. T lymphocytes, as cells originating in the thymus, were discovered in the early sixties⁴⁴, and until the early seventies there was little evidence for their phenotypic or functional heterogeneity. It was not known whether all T cells were functionally equivalent or whether different functions such as help in antibody responses and cytotoxicity were performed by functionally different T cells.

The first unequivocal evidence that T cells represent a collection of functionally different cells was published in 1975³⁰. It was found that effector helper T cells and effector killer T cells can be separated on the basis of their differential expression of CD8 $\alpha\beta$ molecule, at that time identified by anti-Ly2 and anti-Ly3 antibodies³⁰. The Th cells were CD8⁻ (later they were positively identified by expression of the CD4 molecule) and killer T cells were CD8⁺ (later they were found to be CD4⁻). Using the above observation it was then demonstrated that not only treatment of effector T cells, but also pre-treatment of lymphocytes from non-immunized mice by appropriate antibodies selectively abrogated killer and helper function¹². This indicated that

CD4⁺ (CD8⁻) and (CD4⁻) CD8⁺ T cells are generated independently of intentional priming with antigen and thus represent different T cell subsets (lineages).

When in 1983, after a long odyssey, the TCR molecule was finally discovered and found to represent a heterodimer composed of α and β chains^{22, 23, 43}, few people, if any, expected that there may be more species of TCR than one.

Soon, however, another type of TCR molecule, composed of γ and δ chains, was identified^{3, 9, 39, 54}, thus dividing T cells into two major classes: $\alpha\beta$, subdivided into CD4⁺8⁻ and CD4⁻8⁺ subsets, and $\gamma\delta$, the majority of which were found to express neither CD4 nor CD8.

Almost at the same time as $\gamma\delta$ T cells, mature CD4⁻8⁻ i.e. double negative (DN), thymocytes expressing a restricted repertoire of $\alpha\beta$ TCRs were described^{11, 16} which were later found to belong to another major subset of T cells called NKT cells because, in addition to the $\alpha\beta$ TCR, they also express receptors found on NK cells².

In the early nineties, the idea of an extrathymic origin of some T cells obtained strong experimental support^{35, 50, 52} and slowly became accepted. At the same time it was found that, in contrast to thymus-derived CD8 T cells, which express heterodimeric CD8 $\alpha\beta$ molecule, the majority of extrathymically derived $\gamma\delta$ and $\alpha\beta$ T cells residing in the gut epithelium expressed a homodimeric CD8 molecule composed of two α chains²⁰.

Since 1995, the most intensively studied subset of T cells consists of the so-called regulatory CD4⁺25⁺ T cells with suppressive activity⁵⁵.

Heterogeneity of Mature T Cells

The current picture of T cell heterogeneity is presented in Fig. 1. Depending on the anatomical site of origin, T cells can be divided into thymus-derived and extrathymic. The relatively well characterized sites of extrathymic differentiation are so called cryptotopes of the murine gut^{26, 53}.

Thymic and extrathymic T cells express either $\alpha\beta$ or $\gamma\delta$ TCR, thus defining four T cell classes. Thymus-derived $\alpha\beta$ T cells can be subdivided into two subsets of class II MHC restricted CD4⁺ T cells: CD4⁺25⁺ regulatory T cells with suppressive activity and CD4⁺25⁻ with helper activity which, depending on the spectrum of lymphokines secreted as a result of antigenic stimulation, function as so-called Th1 or Th2 cells^{40, 51}. Recently a subpopulation of CD4⁺25⁻ T cells (Ts) with suppressive activity was also described^{1, 58}. Moreover,

Table 1. Chronology of the discovery of major T cell subpopulations

Year	T cell subpopulation	References
1975	CD8 $\alpha\beta$ ⁺ (CD4 ⁻); CD8 $\alpha\beta$ ⁻ (CD4 ⁺)	12, 30
1983	TCR $\alpha\beta$	22, 43
1986	TCR $\gamma\delta$ (CD4 ⁻ 8 ⁻)	3, 9, 39
1987	NKT	11, 16
1991	extrathymic	36, 50
1991	CD8 $\alpha\alpha$	20
1995	CD4 ⁺ 25 ⁺	55

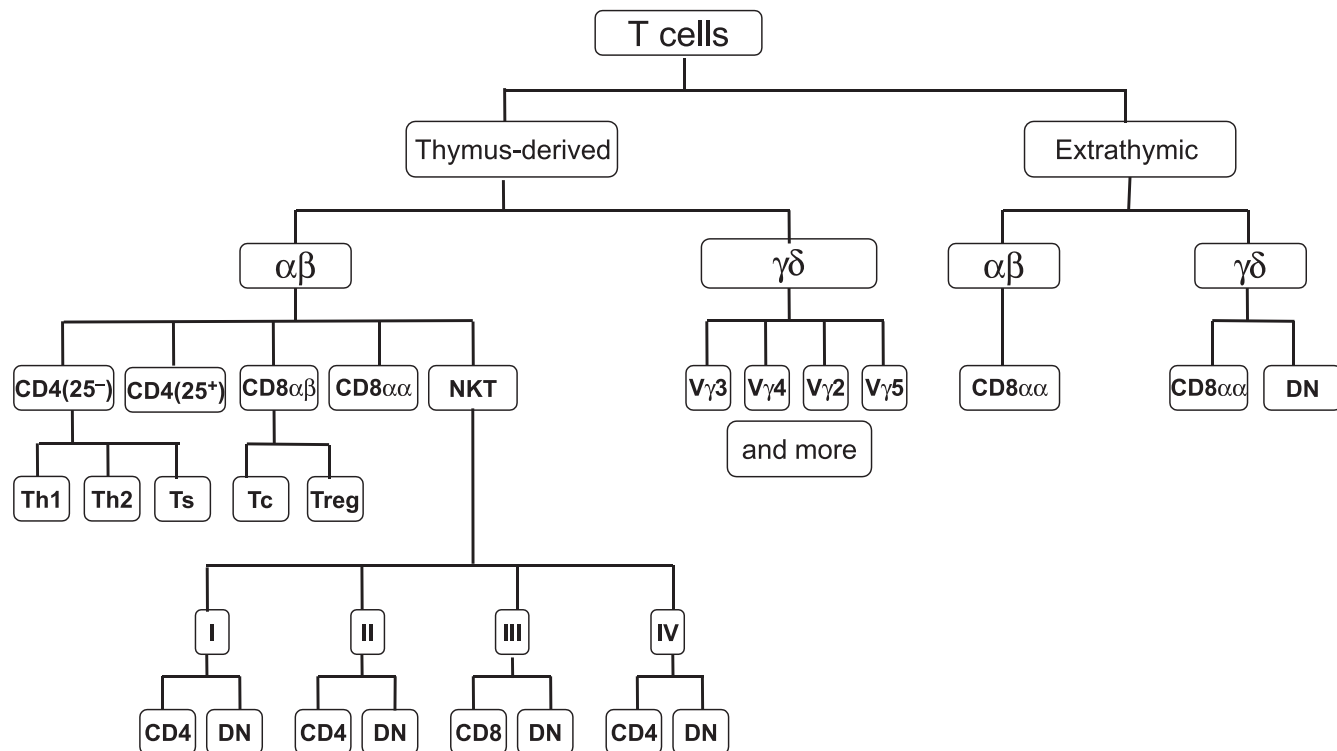


Fig. 1. Heterogeneity of mature T cells. DN – double negative ($CD4^-$, $CD8^-$)

among thymus-derived T cells one can distinguish the following two subpopulations: A – conventional $CD4^-$ T cells expressing heterodimeric $CD8\alpha\beta$ molecule, which can be divided into cytotoxic T cells (Tc) restricted by classical MHC class I molecules and regulatory CD8 T cells (Treg) restricted by the nonclassical MHC class I molecule Qa-1²⁴, and B – intraepithelial (IEL) $CD4^-8^+$ T cells expressing homodimeric $CD8\alpha\alpha$ molecule. The $CD4^-8^+$ IEL T cells can be either class I or class II MHC restricted³⁷. $CD8\alpha\alpha$ T cells residing in gut epithelium may also be extrathymically derived³⁴. Subpopulations of conventional CD4 and $CD8\alpha\beta$ T cells co-expressing $CD8\alpha\alpha$ molecules have also been described^{38, 49}.

NKT cells, the majority of which are restricted by the non-polymorphic class I MHC-like molecule CD1d, and which at the time of discovery seemed to represent a minor and homogenous population, were found to be extremely heterogenous. Phenotypically, one can now distinguish eight subpopulations belonging to 4 subgroups⁴¹. The discrimination of these subpopulations was possible by staining with CD4, CD8, NK1.1 and with tetramers of CD1d molecule bound

with synthetic glycolipid α GalCer, which is recognized by the vast majority of NKT cells.

Ten years ago a subpopulation of IEL T cells with $CD4^+8^+$ phenotype of unknown (thymic or extrathymic) origin was described³⁶ and a recent report suggested the existence of $CD4^+8^+$ mature T cells within human peripheral blood⁴⁵. In both these cases it was not clear whether the CD8 molecule expressed on $CD4^+$ cells was hetero- or homo-dimeric.

Thymus-derived $\gamma\delta$ T cells, in contrast to $\gamma\delta$ IELs which express $CD8\alpha\alpha$ homodimer, are mostly of $CD4^-8^-$ (DN) phenotype, but some minor populations expressing CD4 or CD8 have also been described²¹. Subpopulations of $\gamma\delta$ T cells are mainly described on the basis of their time of appearance during ontogeny, tissue localization and TCR repertoires dominated by preferential use of specific $V\gamma$ segments²¹.

Major Stages of Intrathymic Development of T Cells

The scheme of T cell development in the thymus is shown in Fig. 2. One of the important achievements of

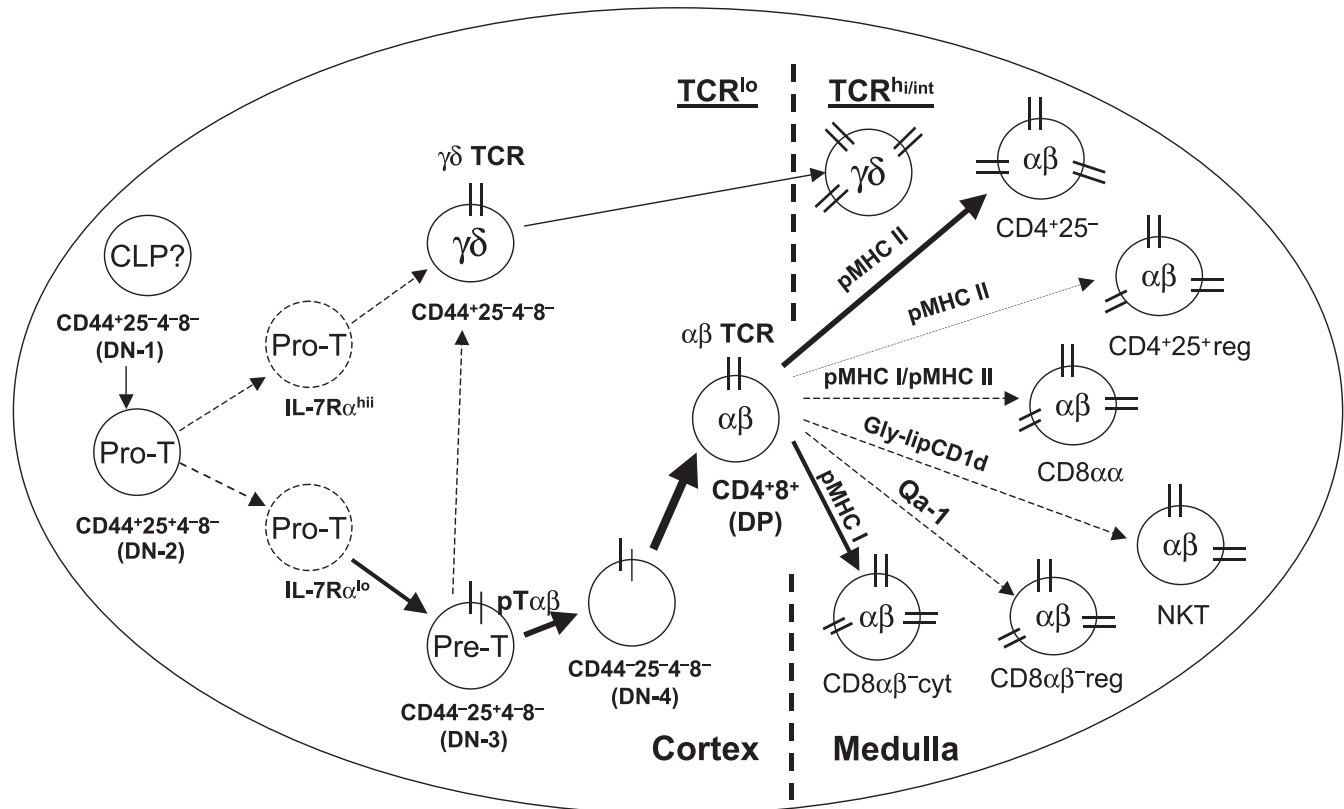


Fig. 2. Development of T cells in the thymus

the research on lymphocyte development during the last 5 years was the identification at the clonal level of the common lymphoid precursor (CLP), which in the adult organism resides in the bone marrow and is able to generate T, B and NK cells³³. In the adult thymus, in the first step of differentiation, the earliest bone marrow-derived precursors, called DN-1, which may be CLP itself but this is unclear (discussed in ref.⁸), express CD25 molecule. Cells at this stage are called pro-T or DN-2 and give rise to cells called pre-T or DN-3. This step is accompanied by downregulation of CD44 expression. Pre-T cells intensively rearrange TCR γ , δ and β genes. Cells that have successfully rearranged TCR γ and δ genes express $\gamma\delta$ TCR and can proceed along the $\gamma\delta$ lineage pathway, which begins either at the pre-T or pro-T cell stage via an intermediate stage expressing a high level of interleukin 7 receptor (IL-7R) molecule²⁷. Cells that have successfully rearranged TCR β genes express pre-TCR (formed by association of TCR β with the invariant pre-TCR α chain¹⁹) and are then able to differentiate along the $\alpha\beta$ lineage pathway, which begins either at the pre-T or pro-T cell stage expressing a low level of IL-7R²⁷.

While we do not know about the existence of intermediate stages between immature and mature $\gamma\delta$ T cells (the former are characterized by a low level of TCR expression and the latter by a high level of TCR expression), the development of mature $\alpha\beta$ T cells from pre-T cells proceeds through at least two additional intermediate stages: DN-4 and CD4⁺8⁺ double positive (DP) (reviewed in ref.³²). The rearrangement and expression of TCR α genes takes place at the DP stage.

The transition from the DN to the DP stage is often referred to as TCR β selection. Until not so long ago, i.e. until the late 80's, the role of DP thymocytes was quite mysterious and no experiment was conducted to exclude the seriously considered possibility that they represent a dead end in the development of T cells and that mature T cells developed from some unidentified precursors (reviewed in ref.³²).

Today the situation is completely different and it is generally believed, although not in every case proven, that all mature, thymus-derived $\alpha\beta$ T cells originate from DP thymocytes: not only CD4 and CD8 $\alpha\beta$ subsets of conventional T cells that recognize antigens in the context of conventional class II and class I MHC pro-

teins, respectively⁶¹, but also CD4⁺25⁺ immunoregulatory suppressor T cells²⁵, $\alpha\beta$ T cells expressing CD8 $\alpha\alpha$ only³⁷, NKT cells¹⁷ and regulatory Qa-1 restricted CD8 $\alpha\beta$ T cells⁵⁹.

In contrast to the intrathymic development, the extrathymic development of T cells, which takes place in the cryptopatches of murine gut generates mostly CD8 $\alpha\alpha$ homodimer-expressing cells and is characterized by the dominance of $\gamma\delta$ lineage precursors³⁴.

One of the most hotly debated issues among researchers studying T cell development concerns the mechanism by which DP thymocytes become committed to the conventional CD4 or CD8 T cell lineages. Considering the fact that despite great effort the issue is not yet settled (see discussion in ref.⁸), the mechanism of DP thymocyte commitment to other lineages may be even more difficult to solve. Until recently the most popular were two models, the instructional and the stochastic/selective. According to the first model^{7, 56, 63}, the signals generated by the ligation of TCR with CD4 coreceptor by positively selecting class II MHC/peptide complexes or by the ligation of TCR with CD8 coreceptor by positively selecting class I MHC/peptide complexes were qualitatively different and resulted in termination of the transcription of CD8 or CD4 co-receptor, respectively. According to the second model^{13, 14}, the ligation of TCR with CD4 or CD8 co-receptor results in random inhibition of transcription of CD4 and CD8 genes, but only those cells which have terminated the expression of the “mismatching” coreceptor, e.g. CD4 in case of a thymocyte bearing a class I MHC-restricted TCR, can differentiate further because only they can receive a survival signal. Yet another model (instructional/default)^{6, 60} postulated that irrespective of the initial signal, mediated by TCR ligated either with CD4 or CD8, DP thymocyte selectively terminates CD8 transcription unless specifically instructed to selectively terminate CD4 transcription. More recently a new “kinetic signaling” model was proposed^{10, 57}, according to which all DP thymocytes are preprogrammed to transiently terminate transcription of CD8 in response to TCR-mediated signaling. Lineage commitment, according to the kinetic signaling model, would occur at the transient CD4⁺8⁻ stage and not at the DP stage and would be determined simply by the persistence or termination of the signal. Persistence of the signal would result in differentiation into CD4⁺8⁻ T cells, whereas termination of the signal would result in co-receptor reversal, i.e. reinitiation of CD8 transcription and termination of CD4 transcription.

Some Important Rules of T Cell Development

The present knowledge about T cell development allows to formulate a number of important rules which seem to underlie this process:

- T cells originate from CLP cells³³,
- receptor Notch-1 plays a vital role in the commitment of CLP to the T cell lineage, both intra- and extra-thymically^{66, 67},
- TCR γ , δ and β but not TCR α gene rearrangements occur at the early DN stages: mainly at pre-T (DN-3), some at pro-T (DN-2)^{18, 27},
- TCR gene rearrangements are necessary for T cell development^{46–48},
- expression of pre-TCR is crucial for $\alpha\beta$ but not $\gamma\delta$ T cell development, both in the thymus and in the gut^{15, 34},
- rearrangements of TCR α genes, in contrast to TCR β genes, are not subject to allelic exclusion^{42, 64},
- DP thymocytes represent the major stage of $\alpha\beta$ TCR repertoire selection, which involves negative selection of thymocytes bearing potentially dangerous autoreactive TCRs as well as thymocytes bearing useless TCR unable to recognize foreign antigens and positive selection of useful thymocytes bearing receptors able to recognize foreign antigens^{29, 31, 65},
- as a rule, intrathymic negative selection of most conventional T cells is induced by agonist peptide/MHC ligands expressed by bone marrow-derived cells and positive selection is induced by non-agonist peptide/MHC ligands expressed by cortical epithelial cells³¹ (exceptions: CD8 $\alpha\beta$ T reg cells selected by Qa-1; CD8 $\alpha\alpha$ and NKT),
- DP thymocytes selected by class II MHC/peptide complexes differentiate into mature CD4⁺25⁻CD8⁻ T cells, whereas DP thymocytes selected by class I MHC/peptide complexes differentiate into mature CD4⁺8 $\alpha\beta$ ⁺ T cells^{28, 31, 62},
- CD4⁺25⁺ suppressor regulatory T cells are generated from DP thymocytes and can be selected by agonist ligands on cortical epithelium²⁵,
- thymus-derived CD8 $\alpha\alpha$ T cells are positively selected by agonist peptides presented on class I or class II MHC molecules³⁷,
- NKT cells with semi-invariant V α 14⁺ TCR are positively selected in the thymus by the MHC class I-like CD1d molecule on cortical thymocytes^{4, 57},
- Qa-1-restricted CD8 $\alpha\beta$ T cells are selected in the thymus by interaction with the Qa-1 molecule on BM-derived hemopoietic cells⁵⁹.

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