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Educational Note

T cell-lineage fate commitment and development

Running title: T cell development

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Abstract

T lymphocytes establish and maintain immune responses. T cell blood precursors under thymic signaling express TCR and CD4 or CD8 co-receptor and differentiate into naïve T cells. This educational note illustrates the major molecular processes that facilitate development of the double negative pre-thymic cell towards a mature T cell. Basic principles of each model, selective or instructive, trying to elucidate the biologic pressures under which T cell fate is determined are presented. The aim is those in need to better understand the basics of T cell fate choice.

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Keywords- T cell; thymus; TCR; CD4; CD8; immunity

I. INTRODUCTION

Immune system relies on T lymphocytes to establish and retain immune responses. T cell progenitors stem from bone marrow, migrate to thymus in order to proliferate, be selected and mature and finally, after specific lineage differentiation, are delivered to periphery. Thymic microenviromental signals promote numerous genetic and molecular processes that eventually lead to T cell development. This educational note summarizes key concepts regarding T cell fate choice.

II. T CELL FATE COMMITMENT

In order to facilitate T cell fate commitment and development towards a T cell phenotype, blood progenitor cells migrate to thymus and receive Notch signals (1). Interplay with Notch ligands found in stromal cells of thymus participates in initiation of pro-T cell maturation program (2). Pro-T cell thymocyte maturation has been partitioned in four differential stages of double negative (DN; $CD4^-CD8^-$) cells, which can be separated by relative expression of CD44 and CD25, as follows: $CD44^+CD25^-$ - DN1, $CD44^+CD25^+$ - DN2, $CD44^-CD25^+$ - DN3, $CD44^-CD25^-$ - DN4(3). DN cells can differentiate into αβ TCR single positive ($CD4^+CD8^-$ or $CD4^-CD8^+$) or γδ TCR cells (4). Early thymic T cell precursor (DN1 stage cell) under constant exposure to Notch ligands develops to a DN2a cell and proliferates. At this phase, T cell lineage fate has not entirely been determined, and loss of Notch mediated signaling pathways has the potential to rearrange the developmental program of the cell towards a NK, dendritic or granulocyte cell commitment. Next, pro-T cells transition to DN2b-stage, in which TCR gene rearrangement is undertaken and full T cell lineage commitment is accomplished, a process characterized by substantial changes in chromatin organization (5), and transformation of genome-wide epigenetic marking (6). By the time of DN3a stage, RAG1,2 protein mediated VDJβ gene rearrangement has been completed and a TCRβ chain has been produced (7). Pairing of that TCRβ chain with a pre-TCRα chain, generated by expression of a non-rearranged locus results in the formation of the pre-TCRαβ pair. At this point, β-selection is facilitated and cells carrying mutations that interrupt the function of stimulated-pre-TCR complex-dependent intracellular signaling are doomed to mutational arrest (8). Cells effectively transitioning intracellular signals consequently advance to DN3b stage. During DN4 stage non-rearranged locus expression of TCR-a is interrupted and recombination of the same locus produces the TCRa chain, thus completing the formation of αβ TCR. Simultaneously, thymocytes induce CD8 and CD4 expression, which in term leads to formation of a double positive ($CD4^+CD8^+$) cell population. Transcription factors and their interactions with chromatin

states driving the T cell fate choice are extensively reviewed by Hosokawa H. et al. (9).

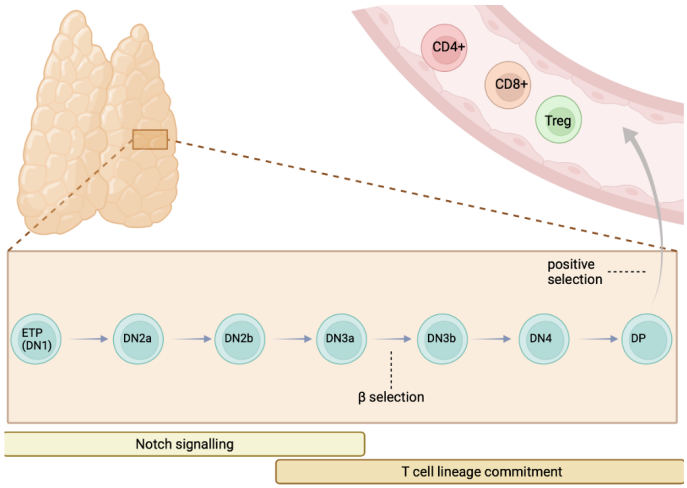

Fig. 1. T-cell lineage development via Notch signaling and T cell lineage commitment

III. POSITIVE SELECTION OF DOUBLE POSITIVE CELLS

Once double positive cells are formed, mechanisms of selection pressures are retained, guaranteeing that only those cells with appropriate functions are permitted to mature and migrate to peripheral tissues (10). The vast majority of double positive thymocytes, express TCRs incapable of self-peptide-MHC complex interactions and subsequently impotent of generating pro-survival signals to sustain cell viability, condemning cells bearing them to a process known as death by neglect (11). On the other hand, high affinity engagement of TCRs to self-peptides provokes sudden apoptotic death, a negative selection procedure securing avoidance of the exacerbation of autoimmunity. TCR interaction with self-peptides in an affinity range between that urging death by neglect and that of negative selection, promotes a process that secures maturation and survival of cells expressing potentially useful TCRs. This process is well-known as positive selection (12). Moreover, double positive cells express both co-receptors CD4 and CD8, whom extracellular domain assists MHC-ligand-TCR interaction and intracellular domain, which relates with protein tyrosine kinase LCK, enhances signals transduced by TCR. CD4 binds specifically to MHC class II, while CD8 to MHC class I. Interestingly, cells expressing TCRs competent to interact with non-MHC specific ligands are not positively selected. As described by Singer A. et al.(13) intracellular levels of LCK are limited and tightly associated to CD4 and/or CD8

molecule function. To promote positive selection adequately intensive TCR signaling requires LCK-assisted signal enhancement. MHC-restricted TCRs, exploiting CD4 or CD8 molecule assistance are able of producing intracellular pro-survival signals, efficient to induce positive selection, whereas non-MHC-restricted TCR bearing cells undergo unavoidably death by neglection. Interestingly, TCR affinity at the higher levels of positive selection encourages clonal deviation, relaying potentially autoreactive T cells towards regulatory (Treg) cell lineage(12). Positively selected population eventually matures in T cells expressing either CD4 or CD8. This process of differentiation depends on MHC class-specific signals.

IV. SELECTIVE AND INSTRUCTIVE MODELS OF T CELL COMMITMENT

The transformation of the thymocyte from double positive to the single positive cell necessitates silencing of transcription of locus for the co-receptor selected to be forsaken and simultaneous genetic events that accompany the CD4/CD8 lineage choice of a T effector type (14). The stochastic selection model dictates that during TCR interaction with self-ligand-MHC complexes, during positive selection of double positive thymocytes, randomly selected locus expression of either CD4 or CD8 is terminated (15). Such a process leads to the formation of a single positive thymocyte bearing a MHC specific class restricted TCR, and a randomly chosen co-receptor, which may match to TCR MCH restriction or may not. A second TCR mediated rescue step guarantees that only maintained cells with a matching co-receptor and TCR are matured and differentiated towards a T cell. Backing for this model has been obtained from co-receptor rescue experiments, in which transgenic co-receptor protein expression rescued T cells bearing co-receptors with inappropriate specificities of MHC-restricted TCRs, signifying that undeniably a second rescue step is taking place (16). However, experimental observation has opposed primary values of the stochastic model (13).

The strength-of-signal instructive model is based on the assumption that TCR specificity induces silencing of expression of mismatching co-receptor. This determines the lineage of double positive cells. More specifically, the cytoplasmic domain of CD4 has been found to bind more LCK than that of CD8 and upon TCR-MHC class II engagement to generate strong signals, while that of CD8 upon TCR-MHC class I interaction to generate weak signals. Relative intracellular signal strength results in inhibited expression of *CD4* or *CD8* gene. Experiments

utilizing chimeric co-receptors consisting of CD8a molecules and the cytoplasmic domains of CD4 set the fundamental principles of strength-of-signal instructive model(17). Expression of engineered CD8-CD4 molecules from MHC class I-restricted double positive thymocytes induced the differentiation of CD4 T cells, that would otherwise—bearing the regular CD8 co-receptor—progress to CD8 T cells. However, experimental work has shown that when ITAMs were altered (in order to evaluate the effect of TCR signaling strength on T cell lineage choice), decrease of signaling intensity did not alter the extent of differentiation towards CD8 or CD4 cells(18). Hence, strength-of-signal model has since been challenged.

Duration-of-signal instructive model could be regarded as a refined version of strength-of-signal model. The core difference differentiating the two is that the first one provisions that duration of TCR stimulation determines the T cell lineage choice. Short TCR stimulation instructs double positive thymocytes to differentiate into $CD8^+$ cells, whereas long TCR stimulation induces $CD4^+$ cell differentiation(19). An explanation of the existence of different duration TCR signals has been attempted to be given by evidence supporting that double positive thymocytes upon TCR stimulation decrease the expression of CD8 co-receptor(20). In case of MHC class I restricted TCR stimulation, CD8 downregulation results in interruption of signaling and in short duration of signals, whereas in case of MHC class II-restricted TCR stimulation, CD8 downregulation does not influence CD4 co-receptor-mediated signaling. Duration-of-signal instructive model also comes with its own drawbacks, and part of its core elements has been also challenged by recent experimental data. Maintaining components of the duration-of-signal instructive model, other models explaining the T cell fate choice, such as the kinetic signaling model have been elaborated(13).

V. CONCLUSION

The processes involved in T cell development and choice of lineage fate have comprehensively been studied. While advances through experimental observations have assisted efforts to uncover the complex mechanisms, in molecular and genetic level, that eventually define whether CD4 or CD8 co-receptor surface expression will prevail, much remain to be explored. Future research to support a definitive model of T cell fate determination is warranted.

AUTHOR CONTRIBUTIONS

SGT drafted the manuscript. DPB revised the manuscript. The artwork was prepared using BioRender under license to DPB. All authors approved the final version of the manuscript.

CONFLICT OF INTEREST

All Authors declare no conflict of interest.

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