REVIEW

# The cellular and molecular mechanism of CD4/CD8 lineage commitment

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**Abstract : A unique feature of αβ T-cell development is the central role played by clonally distributed T-cell receptors (TCR), which are encoded by somatically rearranged gene segments that produce a diverse, non-germline encoded set of receptors. Fate determination in individual T-cells is mediated by ligand-receptor signals that arise from unprogrammed genetic interactions, under conditions in which the relevant ligand concentration and the receptor affinity are not evolutionarily controlled. A precursor T-cell with a TCR that either fails to demonstrate appreciable self-reactivity or binds with high affinity to reasonably abundant self-peptide major histocompatibility complex (MHC)-ligands will undergo apoptosis. In contrast, a precursor T-cell that shows lower affinity to moderately abundant ligands will receive suitable signals for survival and maturation. Recently, we have developed a rapid *in vitro* two-step organ culture system that permits homogeneous populations of non-transformed precursor T-cells to undergo selective commitment to the CD4 or CD8 lineage. Using this model, we have shown that the choice of positively selected ab T-cells between the CD4 helper and CD8 cytotoxic lineages is regulated by the TCR signaling duration in response to self-peptides bound to the MHC. J. Med. Invest. 49 : 1-6, 2002**

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## Overview of thymocytes development

Multipotential progenitor cells receive numerous intracellular signals during the differentiation from the interaction of the cell surface receptors with various types of ligands (1). The biochemical changes induced in the progenitor cells by these interactions alter the expression and function of specific transcription factors, which leads to either the production of cell survival or cell death signals, which result in either the development of a differentiated cell of a particular lineage or the elimination of that cell respectively.

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Thymocyte development also proceeds through an ordered series of proliferation and maturation events that first generates immature T-cells with a pre-T cell antigen receptor complex, followed by the development of mature T-cells with a diverse repertoire of antigen-specific αβ T-cell receptors encoded by somatically rearranged gene segments (2-4). αβ T-cell development is controlled by signals that arise from interactions between the clonally expressed antigen receptor and ligands that consist of self-peptides bound to major histocompatibility complex (MHC) molecules expressed on thymic stromal cells. These signals either lead to continued maturation (positive selection) or to activation-induced cell death (negative selection) (3, 5, 6). The fate of each developing T-cell is thus believed to depend on the strength and timing of the TCR-MHC interaction, in which weak interactions promote positive selection and strong interactions

lead to thymocyte activation and cell death (5, 6).

## Models for CD 4/CD 8 T-cell lineage choice

These same TCR interactions with self-peptide and MHC ligands also dictate the lineage fate of immature $CD4^+CD8^+$ (double positive or DP) thymocytes. Studies have shown that the TCR specificity for either class I or class II thymic MHC molecules ultimately determines whether a T-cell develops into a mature $CD8^+$ cytotoxic T-cell or a $CD4^+$ helper T-cell, respectively (7-9). CD4, which is specific for MHC class II, and CD8, which is specific for MHC class I, are proteins that show peptide-independent, MHC-class specific interactions. It was initially postulated that the CD4/CD8 lineage choice occurred by an instructive mechanism, such that co-engagement of the $\alpha\beta$TCR and the CD8 coreceptor by MHC class I molecules or the $\alpha\beta$TCR and CD4 coreceptor by MHC class II molecules would result in qualitatively distinct signals directing differentiation into the CD8 or CD4 lineage, respectively (5).

Early TCR transgenic mice experiments were consistent with this notion. However, subsequent studies suggested that the match between coreceptor expression and the TCR MHC bias was ascribed to a two-step process that involved an initial stochastic lineage choice upon initial TCR signaling, which led to the loss of either CD4 or CD8 expression. The next step was to determine if the remaining coreceptor was able to participate in ligand recognition with the TCR. Cells with incompatible TCR and coreceptor combinations would die due to a lack of appropriate survival signals at this second maturation step. This view became known as the CD4/CD8 lineage development stochastic/selection model (10-13).

Recent experiments suggested that the observations leading to the competing instructive and selective models could be accommodated by postulating that quantitative differences in TCR and coreceptor signaling were transformed into qualitative differences in cell behavior. Instruction did occur, but not through a mechanism requiring unique biochemical signals from co-engagement of the TCR with either CD4 or CD8. Instead, stronger signals favor CD4 development, whereas weaker signals favor CD8 development (14, 15). These proposals failed to address how the signal "strength" leading to the proper lineage choice could be predictably obtained by T-cells expressing random specificities for MHC class I vs. class II molecules and assumed that identical signals controlled fate restriction and subsequent maturation.

## Duration of TCR signaling and CD4/CD8 T-cell fate choice

Our analysis led us to conclude that a major problem in understanding thymocyte developmental regulation was the inability to accurately control the nature, quantity, and quality of TCR-ligand interactions. Further progress required a model that permitted the manipulation of these parameters, while at the same time, preserved the utilization of physiological ligands and the complex thymic organization. To this end, we have developed a modified version of the reaggregate culture method of Jenkinson and Owens that permits experimental variation in the TCR ligands at early versus late differentiation stages. This system also allows modification of the proteins expressed by the T-cells or the surrounding stromal cells in a quasi-physiological organ culture environment (Fig. 1) (16). The modified two-step reaggregate culture system uses thymocytes expressing AND (MHC class II specific) or HY (MHC class I specific) TCR together with presenting cells with wild-type or mutant MHC loci and various inhibitors, which include antibodies and antisense RNAs. Specifically, $CD4^+CD8^+$ thymocytes from TCR transgenic mice were crossed with RAG-2-/- mice. On a non-selective background, the $CD4^+CD8^+$ thymocytes were stimulated by splenic or thymic dendritic cells (DC) in the presence or absence of specific antigenic peptides for 20 hours (1st step). CD69hi cells were purified and reaggregated with thymic stromal cells (TSC) plus DC and cultured for several days (2nd step). The TCR signal at the1st step does not turn off CD4 or CD8 gene expression, which is assessed by a pronase stripping and re-expression assay. Instead, selective CD4 or CD8 expression is seen on most cells emerging from the second culture step. Using this system, it became possible to manipulate the TCR and other extrinsic signals in each thymocyte differentiation step. We have recently reported that bipotentiality loss by DP thymocytes (lineage commitment) occurs rapidly upon TCR and coreceptor engagement, with the CD4 vs. CD8 choice showing a clear dependence on the duration of effective TCR signaling. A short signal (4 hr) promotes CD8 development, whereas with the same T-cell population and ligand, prolonged signaling (14 hr) leads to CD4 development. Interestingly, although the signaled cells show loss of bipotentiality within this time frame, they do not show

selective silencing of CD4 or CD8 expression if maintained in a culture lacking stromal cells. Thus, lineage commitment can be clearly separated from signals necessary for lineage progression among committed cells. In a second stage culture of committed cells with thymic stroma, phenotypic change and functional maturation does occur (Fig. 2).

These data add substantially to our understanding of thymocyte development in terms of the extrinsic signals controlling lineage specific differentiation ; however, they do not address the more fundamental questions of (i) how in a relatively predictable manner MHC class I vs. class II ligands lead to short vs. long duration signals in most precursor T-cells ; (ii) how TCR signaling differences restrict development potential at the molecular level ; or (iii) how TCR and other signals are integrated to control the lineage specific genetic program that results in CD4 vs. CD8

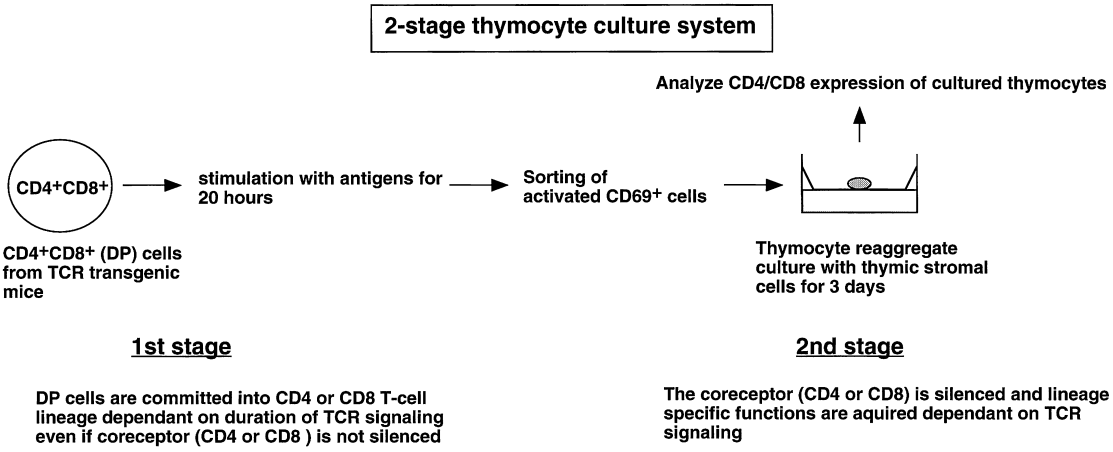


Fig. 1 Scheme of experimental system for examining molecular basis of CD4/CD8 lineage choice
Outline of T-cell development. This figure shows a model of CD4/CD8 T-cell lineage choice.

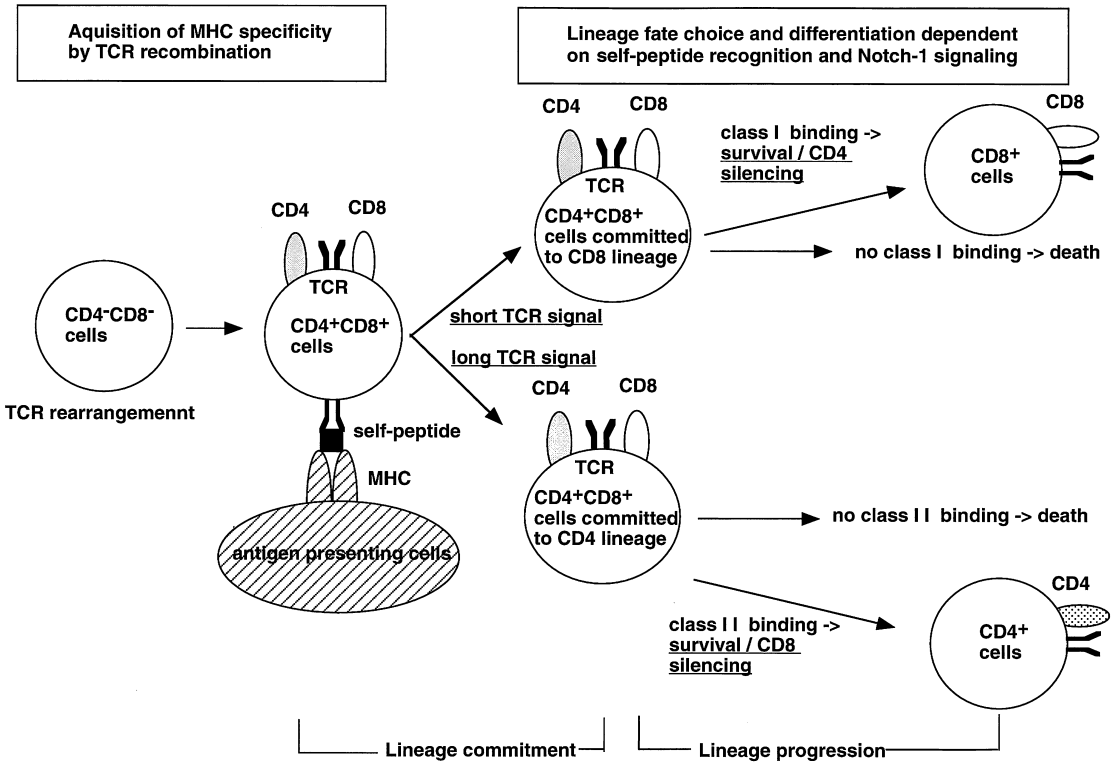


Fig. 2 Scheme of T-cell development
Experimental system for examining the molecular basis of CD4/CD8 lineage choice. This figure shows a two-stage thymocyte culture system. $CD4^+CD8^+$ cells from TCR transgenic mice crossed with rag2 -/- (neutral background) are stimulated with a given antigen for 20 hours in suspension culture. The sorted live $CD69^+$ cells are cultured with thymic stromal cells in the thymocyte reaggregate culture system for 3-4 days. The phenotype or thymocyte cell number are evaluated by flow cytometry.

mature T-cells. Regarding the first issue, it is clear that the same a and b gene segments are used to create the receptors that show preferential binding to self-peptides presented by MHC class I vs. MHC class II molecules. Also, biophysical measurements have failed to detect a systematic difference in ligand binding affinity of MHC class I vs. class II specific TCR. Interestingly, the class I and class II ligand abundance is similar on thymic stromal cells. Therefore, it is difficult to imagine that there is a predictable bias in the TCR affinity interacting with MHC class I vs. MHC class II ligands in the thymus, or even a difference in the available ligand quantity to these TCR. Thus, the distinct duration of the signal origin in response to MHC class I vs. class II ligands is likely to arise from a different source. There is strong evidence that the association of the src family kinase Lck with CD4 is strikingly different from its association with CD8, with the former being much more extensive in DP thymocytes. Based on previous work, the nature of TCR induced proximal tyrosine phosphorylation events is regulated by the extent of co-recruitment of Lck-coupled coreceptors (17). The Lck-deficiency of most CD8 molecules on DP thymocytes would thus favor limited signaling in comparison to CD4 with its high ratio of Lck. Placing the critical distinction between class I vs. class II recognition on the coreceptor acting in concert with the TCR supports the data on the ability of a coreceptor cytoplasmic tail switch to markedly change cell fate, because this is the region of the molecule regulating Lck association. It is also possible that alternation in proximal tyrosine phosphorylation seen when TCR are deprived of effective coreceptor binding is associated with a more rapid desensitization of the receptor pool by phosphatases. This is consistent with the evidence mentioned above that signaling duration is key in the fate decision process.

What molecular events result from long vs. short duration TCR signals and constrain developmental potential (mediate linage commitment) remain unknown. One candidate for controlling the CD4 vs. CD8 decision is MAPK. Interference with MAPK activity limits CD4 but not CD8 development, whereas increased MAPK activity results in CD4 development (18). Recent studies have revealed that ERK directly modifies Lck and changes its susceptibility to SHP-1 binding and inactivation (17). This positive feedback loop plays a dominant role in controlling the effective TCR signaling duration. Thus, existing data on MAPK can also be interpreted as a regulator of proximal TCR signaling. This leaves the entire spectrum of downstream signaling pathways open in terms of their role and relevance to the commitment and progression events. Thus, it will be important to examine both protein modifications and gene expression changes that occur differentially in CD4 vs. CD8 committed thymocytes to determine how TCR signaling differences are converted into developmental potential limitations and the CD4 or CD8 maturation program.

## Notch and CD4/CD8 lineage commitment

In addition to TCR signaling, the general cell differentiation regulator Notch has been examined for its role in this fate decision. Robey *et al*. first proposed that Notch activity plays a critical role in lineage commitment toward CD8, based on results using mice expressing a truncated, active Notch-1 transgene (19). However, Deftos *et al*. have reported that Notch expression prolongs cell survival by upregulating Bcl-2. They concluded that the increased cell survival of $CD4^+CD8^+$ thymocytes in Notch-1 transgenic mice could result in an apparent bias towards $CD8^+CD4^-$ T-cells, based on a similar phenotype in Bcl-2 transgenic mice (20). Neither of these experimental systems has examined separately the role of Notch in both early and late phases of thymocyte selection and differentiation. Based on our evidence for separation between the commitment and progression phases of T cell differentiation, we utilized our two-step culture system to examine the effects of a Notch blocking antibody or expression of a retrovirus encoding anti-sense Notch-1. With both, we found that interfering with Notch activity affects $CD8^+$ but not $CD4^+$ T-cell development (16). The results using the anti-Notch-1 mAb showed that inhibition of Notch activity blocks the $CD8^+$ T-cell development, but does not enhance $CD4^+$ T-cell development. These results suggest that Notch activity contributes only to cell lineage progression committed to the CD8 pathway and not the actual lineage decision process.

Subsequent reports by Wolfer *et al*. showed that Notch-1 conditional inactivated mice do not have any defect in CD4 and CD8 T-cell development, arguing that Notch-1 does not contribute to the lineage decision between CD4 and CD8 T-cells (21, 22). Rather, Notch-1 is involved in the lineage fate choice between T-cells and B-cells (22). Our results are obtained from the analysis of fetal thymocytes. Previous reports indicated there is a clear difference in Notch receptor expression patterns in fetal and adult thymocytes (23). Thus, the discrepancy may be due to the cell

origin. Another possibility is that other Notch receptors contribute to the lineage fate choice between CD4 and CD8 T-cells. Those issues should be clarified by additional Notch gene inactivation studies and the subsequent analysis of the mature T-cells from those studies.

## Conclusion remarks

There are many types of transgenic mice available to evaluate the role of numerous genes in thymocyte development, including CD4/CD8 lineage choice. However, such studies generally do not clarify if the genes regulate lineage commitment, cell survival, or cell differentiation. In order to examine precisely the role of these genes, our two-step thymocyte culture system may be useful to answer these and other questions.

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