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Review

γδ and αβ T cell lineage choice: Resolution by a stronger sense of being

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ABSTRACT

A common bipotent thymocyte precursor gives rise to both lineages of T cells, αβ and γδ. However, the cell intrinsic and extrinsic factors that influence αβ- versus γδ-lineage bifurcation remain controversial. γδ T cells play a unique and vital role in host defense, from maintaining integrity at epithelial and mucosal barriers to their newly defined role as an important innate source of interleukin-17. Although a T cell receptor (TCR)-independent fate choice may take place, emerging data supports a model in which the differential signaling capacity of αβ and γδTCRs play an instructional role in specifying lineage fate, with strength of signal measured by the amount of ERK/MAPK pathway activation. Here we discuss how the interplay between intrinsic TCR signals and cell extrinsic signals provided by Notch and TCR ligands help to assign and support a final lineage fate decision.

1. Introduction

In 1984, amidst the race to clone and characterize the T cell receptor (TCR) subunits, subtractive-cDNA hybridization, which successfully isolated the TCRβ gene [1], was applied by Saito et al. [2] in an effort to identify and sequence the TCRα gene from a murine cytotoxic T lymphocyte clone. The group isolated a sequence that possessed all of the necessary requirements to be the sought after TCRα: the cDNA was expressed and rearranged specifically in T cells, and had similarities to the immunoglobulin variable and constant region genes. However, upon isolation of the human TCRα chain by another group [3], the lack of homology between the human TCRα and Saito et al.'s mouse gene made it apparent that the isolated mouse gene was not the mouse TCRα, but in fact a third type of T cell receptor gene. The accidental discovery of this "non-α gene" [4], later coined the TCRγ chain [5], led to the beginnings of characterizing an entirely novel branch of T cells, the γδ T cell. Soon after the discovery of the γδ T cell, interest quickly shifted to elucidating the mechanism by which developing thymocytes decide between the αβ- and γδ-lineage fate [6–9]. In this review, established and emerging data in favor of the signal strength instructional model, as well as the key molecular players governing this decision, will be discussed.

2. Stochastic versus instructional model in determining αβ- or γδ-lineage fate

In an early attempt to resolve the question of how the αβ- versus γδ-lineage decision is made, a timing-dependent model was proposed, based on observations that γδ T cells were found in the fetal thymus prior to any detectable αβ T cells [9]. The model predicted that during ontogeny, developing T cells are first given an opportunity to develop along the γδ-lineage. However, if no productive γ- and δ-chains are generated, cells can attempt to develop along the αβ-lineage by successfully rearranging their β- and α-gene loci. Thus, all αβ T cells would be considered failed γδ T cells. In contradiction with this model, KN6 γδTCR transgenic (Tg) mice contained normal numbers of αβ T cells, indicating that productively rearranged γ- and δ-chains did not block αβ T cell generation [6]. This was found to be due to a *cis*-acting DNA silencer present in the regions flanking the γ gene, which made the transgenic γ and δ chains transcriptionally silent [6]. Upon removal of this silencer, development of αβ T cells was severely blocked in KN6 γδTCR transgenic mice, suggesting that factors that activate the silencer play an important role in lineage decisions [7]. In light of these findings, a second model of lineage specification was proposed, whereby commitment to the αβ T cell lineage occurs prior to TCR expression, in precursor T cells programmed to repress the expression of the γ-chain by inducing factors that interact with the silencer element. Unlike the timing-dependent model, in which developing T cells are thought to become the lineage that matches the TCR they express, this model proposed that specification is pre-

Abbreviations: DN, double-negative; DP, double-positive; FTOC, fetal thymic organ culture; Id3, Inhibitor of DNA binding 3; IL, interleukin; MHC, major histocompatibility complex; β2m, β_2-microglobulin; Egr, early growth response; ERK, extracellular signal-regulated kinases; MAPK, mitogen-activated protein kinase; TCR, T cell receptor; tet, tetramer; Tg, transgenic; ZAP-70, Zeta-chain-associated protein kinase 70.

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determined [6]. Despite some of the findings in these studies now being dated by more recent discoveries, their importance is maintained, as they pioneered the ideas for two opposing models for αβ- versus γδ-lineage bifurcation, and initiated a longstanding debate on a topic that still remains relevant today.

These two proposals to explain the underlying mechanisms governing αβ- and γδ-lineage specification have evolved to present day as the stochastic model and the instructional model. Evidence has emerged in support of both [10–14]. The stochastic model states that early thymocytes are pre-committed to a lineage prior to TCR rearrangement and expression. In this case, the role of the TCR is only to provide survival signals to cells whose TCR matches the lineage pre-selected by the cell, and not to direct lineage choice. A broader interpretation posits that pre-commitment is enforced by factors that favor γ and δ over β gene rearrangements and/or expression. Compared to the stochastic model, the instructive model suggests a far more involved role for the TCR, whereby the lineage adopted by the developing T cell is dependent on the TCR expressed. Specifically, the instructive model proposes that distinct signals are produced from the γδTCR and pre-TCR, which differentially instruct cells to adopt the appropriate cell fate: the fate which matches the TCR from which the signal is derived.

3. Point of lineage divergence

To begin deducing which of the two proposed models is correct, and to further elucidate the key molecular factors involved in fate decision, the precise point of αβ- versus γδ-lineage divergence needed to be determined before the experimental data could be accurately interpreted. In the mouse thymus, T cell development within the early subset of $CD4^-$ $CD8^-$ double negative (DN) thymocytes is typically further characterized into four subgroups, DN1 to DN4, based on their expression of CD25 and CD44 [15]. αβ- and γδ-lineage cells arise from a common DN T cell progenitor [14,16,17], in which TCRβ, TCRγ and TCRδ-chain rearrangements have been initiated [18,19]. Rearrangements are complete by the DN3 ($CD44^-$ $CD25^+$) stage, wherein cells are subjected to a selection process that ensures the survival and further differentiation of cells that have generated a productive TCR [20–22]. DN cells that successfully rearrange their TCRβ chain, and express it together with pTα to generate a pre-TCR complex [23,24], progress along the αβ-lineage to become $CD4^+$ $CD8^+$ double-positive (DP) and subsequently $CD4^+$ or $CD8^+$ single-positive (SP) cells [25]. Alternatively, DN cells that produce a γδTCR, remain as DN but down-regulate CD25 expression, and progress along the γδ-lineage [26,27]. In this maturation process, γδ T cells decrease CD24 expression and express a set of genes, termed the γδ gene expression profile, not found in αβ-lineage DP cells [28–30].

If the TCR plays a role in lineage commitment, successful rearrangement and expression of the appropriate TCR needs to precede the point of lineage divergence. In accordance with this, the αβ versus γδ divergence point was found to be after TCRβ, but before TCRα, recombination [31]. To more precisely define the stage of αβ- versus γδ-lineage bifurcation, the lineage potential of DN2 and DN3 cells was recently assessed using an *in vitro* clonal assay [32,33], in which single cells were deposited into individual wells containing OP9-DL1 cell monolayers [34]. Lineage divergence was observed to occur between the late DN2 to DN3 developmental stages, as many DN2 cells were bipotent, while few DN3 cells gave rise to both progeny [34]. Interestingly, a subsequent study looking at the effect of γδTCR expression on lineage decision noted that TCRγδ$^+$ $CD25^+$ thymocytes differentiated into DP cells quite efficiently, while only a few TCRγδ$^+$ $CD25^-$ $CD24^{hi}$ cells and no TCRγδ$^+$ $CD25^-$ $CD24^{lo}$ cells adopted the αβ-lineage fate [35]. This indicates that TCRγδ expression does not prevent immature thymocytes from adopting the αβ-lineage fate, but maturation of TCRγδ-bearing cells, marked by CD24 down-regulation, does mark cells as γδ-lineage cells. Considering that decreased levels of CD24, and not TCRγδ expres-

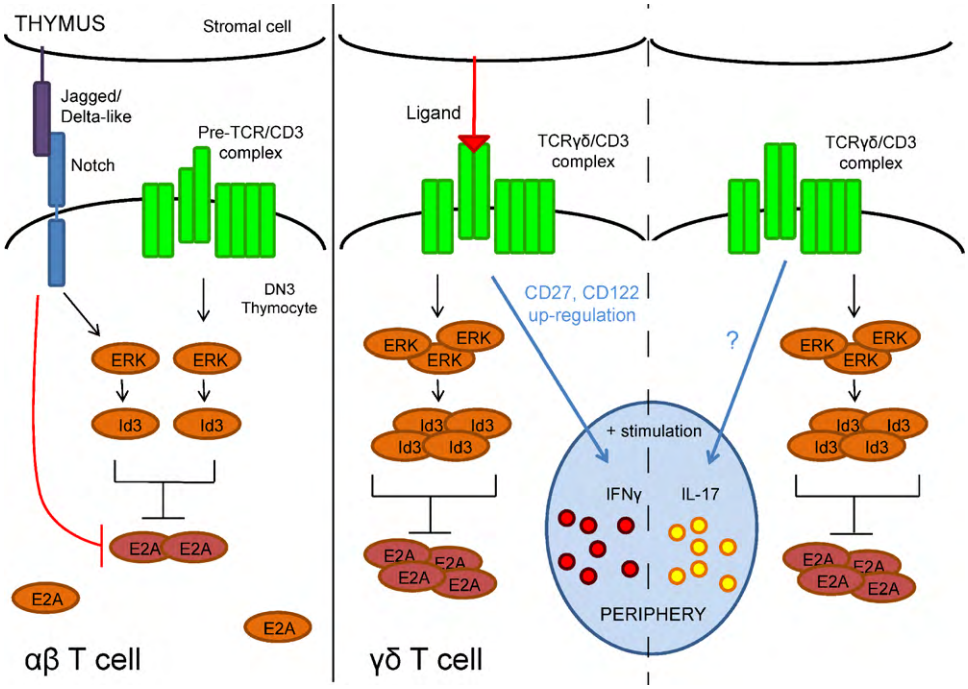

Fig. 1. A schematic overview, based on the strength of TCR signal, of the αβ versus γδ-lineage fate decision made by a DN3 thymocyte. Differentiation into an αβ or γδ T cell is dictated by TCR signal strength delivered to DN3 cells at the point of lineage commitment, as measured by the level of ERK/MAPK pathway activation and Id3 induction. The requirement for Notch signaling at this stage of development depends on the magnitude by which the TCR signals can inhibit E2A. Strong signals produced by the TCRγδ induce high levels of Id3 that allows for further maturation in the absence of Notch signals, while weak signals produced by the pre-TCR require help from the Notch signaling pathway to inhibit E2A to a level necessary for successful β-selection. Furthermore, TCR-ligand engagement is not be required for pre-TCR signaling, and while it may not be necessary for all TCRγδ selection, it may affect the polarization of γδ T cells to IFNγ secretion versus IL-17 production.