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αβ versus γδ lineage choice at the first TCR-controlled checkpoint

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Abstract

αβ and γδ T-cells develop in the thymus from a common precursor. Although lineages initially were defined by the type of TCR they express, it soon became clear that the TCR type per se does not play a deterministic role in the lineage decision, since in various transgenic and knockout models, as well as in a small fraction of cells in wt mice, the TCRγδ can drive the differentiation of αβ lineage cells and the TCRαβ can drive differentiation of γδ lineage cells. Thus until recently it was unclear what determines lineage choice and at which stage the two lineages diverge. Recent observations suggest that TCR signal strength determines lineage fate and that lineage choice is made at or shortly after the first TCR-controlled checkpoint. While it is clear that the decision between αβ and γδ lineages is made at the first TCR controlled checkpoint and the αβ sublineages split off later, it is less clear whether γδ sublineages divert already at the first TCR controlled checkpoint or later. Recent experiments support the former view.

Introduction

The adaptive immune system in all studied jawed vertebrates consists of three lymphocyte types: B-cells, αβ T-cells and γδ T-cells. Whereas B-cells separate early on in development, αβ and γδ T-cells share a large portion of their developmental paths.

αβ and γδ lineages were initially defined on the basis of the T-cell receptor (TCR) expression. In wt mice TCR expression correlates with distinct molecular programs initiated in developing T-cells. CD4/8 double negative (DN) thymocytes initiate rearrangements of three of the four TCR loci – *Tcrb, Tcrg and Tcrd*. Productive rearrangement of *Tcrb* leads to its expression in a complex with the invariant pre-Tα chain (pre-TCR), and pre-TCR signaling results in *Tcrb* allelic exclusion and is followed by a burst of proliferation, progression to the CD4/8 double positive (DP) stage, silencing of *Tcrg* expression, rearrangement of the *Tcra* locus (which leads to the deletion of *Tcrd* found within *Tcra*) and, finally, expression of the αβ TCR. Cells that productively rearrange *Tcrg* and *Tcrd* loci and express the TCRγδ receptor likewise undergo

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While this article was in press interesting findings regarding PLZF-expressing γ-δ T cells were published [Alonzo ES, Gottschalk RA, Das J, Egawa T, Hobbs RM, Pandolfi PP, Pereira P, Nichols KE, Koretzky GA, Jordan MS, et al.: Development of Promyelocytic Zinc Finger and ThPOK-Expressing Innate γδ T Cells Is Controlled by Strength of TCR Signaling and Id3 3. J Immunol 2009.]. The authors demonstrate that PLZF+ TCRgd+ cells coexpress ThPOK - a transcription factor required for CD4 T cell differentiation and induced by relatively strong TCR signaling in these cells. In addition they show that Vγ1+ cells that accumulate in Id3−/− mice are indeed PLZF +Vγ1Vδ6.3 cells as we hypothesized here. Finally, they demonstrate that certain mutations in SLP-76 lead to an increase in PLZF +Vγ1Vδ6.3 cells - similar to the increase observed in Itk−/− and Id3−/− mice.

a burst of proliferation [1], but become functionally mature without progression through the DP stage. In development, execution of a molecular program better defines a lineage than expression of a single receptor. Therefore, progression through DP or lack thereof is widely used as the distinction between αβ and γδ lineages especially since over time it became clear that correspondence between the type of TCR expressed and the developmental history of a cell is not always perfect.

Thymocytes in most TCRαβ transgenic mice express the TCR prematurely at the DN stage. An abnormal population of TCRαβ$^+$ cells can be found in many TCR transgenic strains. Like γδ lineage cells, they exhibit a CD4$^-$CD8$^-$ or CD4$^-$CD8αα$^+$ phenotype, avoid rearrangement of the endogenous *Tcra* locus, and are capable of fast effector responses [2,3]. Although it was suggested that these cells might have progressed through the DP stage and thus corresponded to innate-like αβ lineage cells (e.g. CD8αα IELs) in wt mice, [4] it was later demonstrated by fate mapping experiments that the majority of these cells did not progress through the DP stage and belonged to the γδ lineage [5]. Early TCRαβ expression can likewise happen, albeit rarely, in wt mice where rearrangement of *Tcra* genes in DN cells can be driven by the TCR delta enhancer [6]. When later rearrangements of *Tcra* are blocked by conditional deletion of *Rag2* at the DP stage, substantial numbers of TCRαβ$^+$ γδ lineage-like T-cells can still be found in the periphery. However, they fail to compete with wt cells in mixed bm chimeras [7] – a result that conforms to fate-mapping experiments which showed that virtually all TCRαβ$^+$ cells in wt mice, including CD8αα IELs [8] and NKT-cells [9], progressed through the DP stage and thus were bona fide αβ lineage cells.

Conversely, in mice that can only express TCRγδ, such as TCRβ$^{-/-}$ [10,11] and pTa$^{-/-}$TCRα$^{-/-}$[12] mice, a substantial number of DP cells can still be found. A small population of γδTCR-driven DP cells can likewise be detected in wt mice [10]. Also a *TCRαβ* transgene can support the development of DP cells in the absence of pre-TCRα [12]. TCRαβ and/or TCRγδ-driven αβ T-cells at the periphery of pTα−/− mice seem to be functionally competent as these animals do not show any signs of immunodeficiency under standard specific pathogen-free conditions (HvB, unpublished observations). Thus, whereas the pre-TCR seems to drive the development of αβ lineage only, both TCRγδ and early expressed TCRαβ are compatible with either of the lineage fates (Figure 1).

Evolution of αβ and γδ T cell lineages

All four TCR loci are found in every studied species that expresses Rag, from cartilaginous fish to mammals [13], suggesting an important and persistent contribution of both αβ and γδ T cells to the evolutionary fitness of jawed vertebrates. The stages of thymocyte development, however, are not well defined in species other than rodents. Nevertheless, a stage corresponding to DP cells –a hallmark of the αβ lineage–has been described in birds [14] and amphibians [15], suggesting its evolution at least 350 million years ago (Figure 2). In addition, genomic analysis of the *TCRα/δ* locus in teleost fish reveals that due to RAG-mediated inversion only one of the chains can be expressed in one particular T cell, implying a more ancient origin of the two lineages [16]. The presence in teleosts of CD4 and CD8 co-receptors [17] provides further evidence for two T cell lineages early in the evolution of adaptive immunity.

In contrast, the pre-TCR is a relatively recent acquisition (Figure 2). Careful computational analysis of vertebrate genomes did not reveal pre-TCRα analogs outside the mammal class (MG, unpublished observations). The pre-TCR represents an adaptation which increases the efficacy of αβ lineage selection as well as TCRαβ diversity. We speculate that in other jawed vertebrates, early steps of αβ lineage differentiation were driven by the γδ TCR and/or early-expressed αβ TCRs–as in pre-TCRα−/− mice. In this view, TCRγδ-driven and early expressed

TCRαβ-driven selection of αβ lineage cells in mice can be considered as vestiges of once mainstream developmental paths.

Role of TCR

Albeit not in a deterministic way, the class of the TCR can clearly influence lineage decision, as in wt mice the majority of TCRγδ+ precursors chose the γδ, and pre-TCR expressing precursors the αβ lineage. Two elegant studies suggested that TCR signal strength rather than TCR class determines lineage choice [18,19]. Attenuation of TCR signals in *TCRγδ* transgenic mice by *lck* deficiency [19], or *CD3ζ* hemizygosity [18] led to an increase of the DP compartment. A boost in TCR signal strength such as transgenic expression of CD3ζ and CD5 hemizygosity or deficiency -- led to a decrease in DP thymocytes, accompanied by an increase in absolute numbers of TCRγδ+ DN cells [18]. Consistent results came from mice transgenic for the KN6 TCR [19,20] which recognizes β2-microglobulin-dependent MHC class Ib molecules T10 and T22 [21–23]. *β2m*$^{-/-}$*Rag2*$^{-/-}$KN6 mice showed a decrease in mature γδ cells accompanied by a dramatic increase of the DP compartment (which was virtually absent in the presence of β2m) [19]. These studies demonstrate an important role of TCR signal strength in αβ/γδ lineage choice.

TCR targets

The signal strength model implies a molecular switch downstream of the TCR. Increased Erk phosphorylation in TCRγδ+ thymocytes (when compared to pre-TCR expressing cells) [18] and in KN6 cells developing in the presence of the ligand [19] suggested the involvement of the MAPK pathway. This led to identification of Egr family members and their target Id3, a negative regulator of E protein function [24], as potential players in lineage choice [19] (Figure 3). Indeed, Egr1, Egr2, Egr3 and Id3 were up-regulated in γδ lineage cells [19].

Overexpression of Egr1 interfered with αβ lineage development both in culture [19] and *in vivo* [25] as judged by decreased numbers of KN6 transgenic DP cells developing in the absence of the ligand. Ligand-driven maturation of KN6 TCR transgenic γδ lineage cells was defective in *Id3* $^{-/-}$ animals as judged by their phenotype and impaired ability to proliferate and produce IFNγ upon stimulation [25]. Even more strikingly, *Id3* deficiency led to the appearance of DP cells in KN6 mice in the presence of the ligand. Moreover, overexpression of *Id3* in *Rag2*$^{-/-}$ DN3 cells was sufficient to confer the ability to produce IFNγ in the absence of the TCR [25]. Thus, Id3 induction by a strong TCR signal, possibly mediated by Egr activity, seems to be an important switch favoring the development of the γδ over the αβ lineage.

Accordingly, non-TCR-transgenic *Id3*$^{-/-}$ mice exhibited a drastic decrease in Vγ4 cells in the spleen and Vγ5 DETCs in the skin [25] (here and below Vγ nomenclature is used as suggested by Heilig and Tonegawa [26]). However, the overall number of TCRγδ^{+} thymocytes was dramatically increased in these mice, at least in part due to an increase in the Vγ1 population [25,27]. These cells were functionally competent as judged by their ability to produce IFNγ upon stimulation [27]. Somewhat enhanced *Tcrg* rearrangements in Id3$^{-/-}$ thymocytes [27] or an observation that Id3 can play a role in the deletion of highly autoreactive cells [25] may explain this phenomenon. Therefore, γδ T cells can be subdivided into Id3-dependent and Id3-independent subsets.

Interestingly, γδ T-cells that accumulated in Id3$^{-/-}$ mice exhibited an activated NKT-like phenotype (M. Verykokakis, MD. Boos, BL Kee, ThymUS 2008 conference abstract book, 2008) – reminiscent of the Vγ1Vδ6.3 subset present in wt mice [28]. Vγ1Vδ6.3 cells require the transcription factor PLZF for their functional maturation [29] as do αβ lineage NKT-cells [30,31]. Although the ligand (if any) for Vγ1Vδ6.3 TCR is unknown, both PLZF [29] and the NKT-like phenotype [32] can be induced by TCR cross-linking in immature polyclonal γδ

thymocytes, suggesting that Vγ1Vδ6.3 cells may indeed receive a strong TCR signal *in vivo*. It is thus possible that some of these cells receive a strong enough signal resulting in deletion but can be rescued by *Id3* deficiency. Increased numbers of Vγ1Vδ6.3 cells were also observed in *Itk*$^{-/-}$ mice [33,34]. As Itk is a Tec family kinase involved in TCR signaling this may also indicate a rescue of these cells from deletion by attenuation of TCR signaling.

Interestingly, another *Id* family member – *Id2* –which plays a role in NK cell development [35] and is expressed at high levels by NKT-cells (www.immgen.org, [36]) -- was shown to be a direct target of PLZF in myeloid cells [37]. If the same is true for NKT-cells and/or Vγ1Vδ6.3 cells, PLZF function may at least in part be mediated by this Id3 homolog.

The major function of Id proteins is negative regulation of basic helix-loop-helix E-protein activity. E-proteins need to form hetero- or homodimers to function as transcription factors. Helix-loop-helix Id proteins can dimerize with E-proteins but lack the basic DNA binding region and thus prevent their binding to DNA [24]. E-proteins are required to enforce the β-selection checkpoint as E2A deficiency allows progression of thymocytes incapable of TCR signaling to the DP stage [38]. The DP stage is a hallmark of αβ lineage differentiation and thus differential inhibition of E-protein activity may be required for the development of αβ and γδ lineages. In fact it was suggested that the lineage fate is determined by the level of this interference – with incomplete inhibition through weak TCR signals leading to αβ lineage and more complete inhibition through stronger TCR signals - to γδ lineage commitment [25] (Figure 3).

Instruction vs. selection

While the above clearly establishes a role of TCR signal strength in lineage choice it does not distinguish whether TCR signals directly instruct lineage fate or merely confirm the choice already made by another mechanism. For instance, in the KN6 system [19] the presence of a ligand could simply delete DP cells rather than divert thymocytes to the γδ lineage. Such model could be supported by the observation that in *Id3*$^{-/-}$*b2m*$^{+/+}$KN6 mice, the accumulation of DP cells is accompanied by decreased apoptosis of αβ lineage cells [25]. Here the *Id3* deficiency could merely rescue DP cells from deletion as it might do in case of γδ lineage cells with high affinity for the ligand [25].

In fact some experiments could suggest that lineage choice is made before TCR expression. DN2 cells (a DN stage prior to TCR expression) can be subdivided on the basis of the level of IL-7Rα. IL-7Rα^{hi} cells gave rise to higher proportion of γδ lineage cells than IL-7R low/negative cells [39]. Although this bias may suggest precommitment, it can be explained by a higher frequency of *Tcrd* rearrangements in IL-7Rα^{hi} cells [39]. DN2 cells are also heterogeneous in the expression of sox13 – a transcription factor implicated in γδ T-cell development [40]. However, neither expression of sox13 by by all TCRγδ^{+} cells, nor its cell-intrinsic role was established so far. In fact, *sox13*$^{-/-}$ mice exhibited gross developmental abnormalities [40] and thus a cell autonomous role of *sox13* in γδ T-cell development is questionable. Moreover, we [32] and others [25] demonstrated that *sox13* was not up-regulated in some populations of γδ T-cells. The undefined role and expression pattern of *sox13* in γδ T-cell development makes the interpretation of its variable expression in DN2 cells difficult.

An experiment that can ultimately discriminate between instructive and selective roles of TCR signaling in lineage choice must be performed with single cells, since *in vivo* and in bulk cultures a contribution of cell death cannot be ruled out which might obscure the interpretation of lineage fate experiments. In one such study, single DN2 and DN3 precursors were plated on an OP9-DL1 feeder layer which is able to support both αβ and γδ lineage differentiation [41]. Most DN2-derived clones contained both TCRαβ^{+} and TCRγδ^{+} cells, whereas DN3 cells gave rise exclusively to clones with a single TCR type. The authors concluded that lineage

commitment occurs at the DN2–DN3 transition – and therefore prior to TCR expression. However, the more likely explanation is that the majority of DN3 clones was derived from proliferating DN3b cells which already succeeded in the expression of a TCR [42] – and thus it is not surprising that these clones were 'committed' in terms of TCR expression. Also, since the TCR type does not play an absolutely deterministic role in lineage choice analysis of CD4/CD8 expression must be performed to address the actual lineage fate.

To address the possibility of precommitment we studied the lineage potential of DN3b TCR-expressing thymocytes [42] in the OP9-DL1 coculture system at the single-cell level [32]. We demonstrated that TCRγδ$^+$ DN3b cells, which can give rise to both αβ and γδ lineages, developed only into the γδ lineage when they received a strong signal from the TCR. In particular, the progeny of single TCRγδ$^+$ DN3 cells that developed into the αβ lineage were diverted to the γδ lineage when a strong TCR signal was provided by TCR cross-linking. From this we conclude that commitment to αβ and γδ lineages occurs after TCR expression and is instructed by TCR signals [32].

Role of Notch signaling

Early studies suggested that Notch signaling might directly dictate γδ/αβ lineage fate [43,44]. However over time it became clear that it cooperates with TCR signaling in this process. After TCR expression, pre-TCR- and TCR-expressing cells have different requirements for Notch signaling. Whereas pre-TCR-expressing cells absolutely require Notch ligands of the Delta-like family to survive and progress to the DP stage, TCRγδ or TCRαβ expressing cells do not require them for γδ lineage differentiation, although Notch signaling can increase their proliferation [41,42,45]. The relative independence of γδ lineage cells on Notch signaling was shown to rely on Id3 activity [25]. It was suggested that in αβ lineage cells, cooperation between a Notch signal and a weak TCR signal might be required for sufficient inhibition of E-protein activity [25] (Figure 3). In fact a strong Notch signal was crucial in favoring αβ lineage development from TCRγδ- or TCRαβ-expressing precursors [45]. Thus Notch signaling is required for αβ lineage development but is dispensable for γδ lineage differentiation.

The exact role of Notch in αβ lineage development is unclear. Although Notch signaling is required for survival of DN3 cells [46], whether its role is restricted to survival or whether it is also required for proliferation and differentiation to the αβ lineage remains uncertain. An attempt was made to address this question by compensating the survival defect using constitutively active PKC – PKCαCAT [47]. Retroviral transfection of *Rag*$^{-/-}$ thymocytes with PKCαCAT somewhat increased the yield of cells cultured on OP9-GFP monolayers. Although PKCαCAT was sufficient to drive *Rag*$^{-/-}$ cells to the DP stage on an OP9-DL1 monolayer, no DP cells were found on OP9-GFP monolayer. Whether the increase in the yield on the OP9-GFP monolayer was due to increased survival, proliferation, or both, is unclear [47]. It was also suggested that human αβ cells after β-selection required Notch for their proliferation but not differentiation [48]. Thus the exact role of Notch in αβ lineage development remains unclear.

Counting the lineages at the branch point

The αβ versus γδ lineage decision is frequently considered to be a binary choice –the cell first makes a decision between the αβ and γδ lineage and only then chooses its sublineage fate. This view implies that two sublineage-uncommitted progenitors arise shortly after TCR expression – one for αβ and one for γδ.

This assumption seems to be reasonable for αβ cells. Indeed, αβ T-cell differentiation is accompanied by a series of characteristic molecular events such as coordinated upregulation of CD4, CD8α and CD8β, rearrangement attempts of the *Tcra* locus leading to deletion of

Tcrd genes, *Tcrg* silencing [49], and induction of the RORγt transcription factor [8]. At least some of these events (upregulation of CD4, CD8α, CD8β, RORγt [8]) happen in all αβ lineage cells – implying a common molecular program.

The same does not apply to γδ lineage cells. This lineage is defined merely by the lack of progression through the DP stage and lack of *Tcra* rearrangement and hence maintenance of the *Tcrd* loci. Although several other molecular markers, including ICER, Rgs1, Nur77 family members [50] and sox13 [40] were suggested as γδ lineage markers most of them seem to mark only a fraction of γδ T-cells [25] [32] and none was shown to be expressed by all γδ T-cells.

In αβ lineage development the common steps are driven by the pre-TCR, whereas the distinct characteristics of αβ sublineages appear later, when αβ TCRs are assembled. In the γδ lineage there is no 'pre-TCR equivalent' and it is likely that a cell receives all signals provided by the γδ TCR immediately after its expression.

Thus it is possible that a common molecular program for all γδ lineage cells does not exist – and the lineage choice soon after TCR expression is made between one αβ and several γδ lineages (Figure 4). Differential requirement for Id3 is consistent with this scenario. Importantly, Id3 expression is a relatively proximal consequence of TCR signaling, as a strong increase in Id3 mRNA can be detected as early as 45 minutes upon stimulation [51]. Although this observation does not exclude a possibility that all γδ lineage cells share some common molecular program – it drastically limits the time frame for its execution. Although TCR signal strength was shown to be an important factor for γδ lineage commitment in several different systems [18,19,32] - it still remains to be seen whether other mechanisms might play a role for some of the γδ sublineages.

Role of γδTCR ligands

T10/T22-specific γδ T-cells, which constitute about 5% of total γδ T-cells in spleen and thymus of a wt mouse [52], represent a unique case where the self specificity of a γδ TCR has been formally proven. If the signal strength model is correct for all γδ lineage cells, other γδ T-cells may also be selected by agonist ligands. In support of this hypothesis, development of the canonical skin Vγ5Vδ1 T-cell population required the expression of the Skint1 receptor on thymic stroma [53,54]. The authors suggest that it may be a ligand for the Vγ5Vδ1 TCR. However the evidence for this is indirect – a block in maturation of Vγ5Vδ1 in thymus organ cultures lacking *Skint1* can be relieved by TCR cross-linking [53]. Thus whether Skint1 is the TCR ligand, a part of the ligand, a co-stimulatory molecule, or an accessory molecule required for the expression of the ligand is unclear. Other indirect evidence for agonist selection comes from the observation that the transcription factor PLZF, which is expressed by Vγ1Vδ6.3 cells, can be induced in polyclonal immature γδ thymocytes by TCR cross-linking [29]. Interestingly, both recombinant Vγ1Vδ6.3 and Vγ5Vδ1 TCRs as well as the Vγ6Vδ1 TCR can bind to various murine cell lines, which may indicate an interaction with a TCR ligand [55]. Alternatively, a γδTCR could signal in a ligand-independent fashion as is the case for pre-TCR signaling [56, 57] as some γδ TCRs can spontaneously dimerize on the cell surface [52].

Unlike in the KN6 system, TCR non-transgenic mice on a wt or *β2m*$^{-/-}$ background have comparable numbers of T10/T22 specific γδ T-cells [52] suggesting that positive selection by a ligand may not be absolutely required for γδ lineage differentiation. However, one cannot exclude the existence of an alternative, non-*β2m* dependent ligand which cross-reacts with T10/T22-specific TCRs. An important difference between *Rag*$^{-/-}$KN6 mice and wt animals is the presence of large numbers of pre-TCR-expressing thymocytes in the latter. It was shown that, in the presence of pre-TCR expressing precursors, αβ or γδ TCR-expressing cells (which under non-competitive conditions can generate DP cells relatively efficiently) are much less efficient in progression to the DP stage and retain a TCR+DN phenotype – even though it is

unclear whether they become functionally mature γδ lineage cells [45,58]. Such 'displacement' from the αβ lineage by inefficient competition with pre-TCR-expressing cells may be an additional mechanism contributing to γδ lineage differentiation. This competitive disadvantage may explain why in KN6 mice the *β2m* deficiency leads to a 5-fold decrease in mature γδ T-cells whereas in non transgenic mice it does not significantly affect the numbers of T10/T22 specific γδ T-cells. Whether or not the strong signal required for γδ lineage differentiation depends on the presence of a ligand is still not clear.

Conclusion

Recent work from many groups convincingly demonstrated that TCR signal strength determines αβ versus γδ lineage choice: a strong TCR signal results in γδ and weak signal in αβ lineage commitment. Single cell experiments show that the TCR instructs rather than confirms lineage choice. The molecular mechanism downstream of TCR signaling which may be involved in this decision is starting to unfold – however, many questions remain open. For instance it is unclear how the lineage choice of Id3-independent γδ lineage cells is mediated. It remains to be seen whether the role of Id3 in γδ lineage differentiation solely relies on its ability to counteract the function of E-proteins and if so – which downstream targets are involved. It is likewise unknown how different levels of E-protein inhibition translate into different lineage fates and whether this is the only mechanism that affects lineage commitment. Whether or not the strong TCR signal which instructs γδ lineage commitment always relies on the presence of a ligand is not known. Finally, it remains to be seen whether the TCR signal strength is the only mechanism that determines lineage fate or whether some γδ lineages require additional mechanisms to choose their fate.

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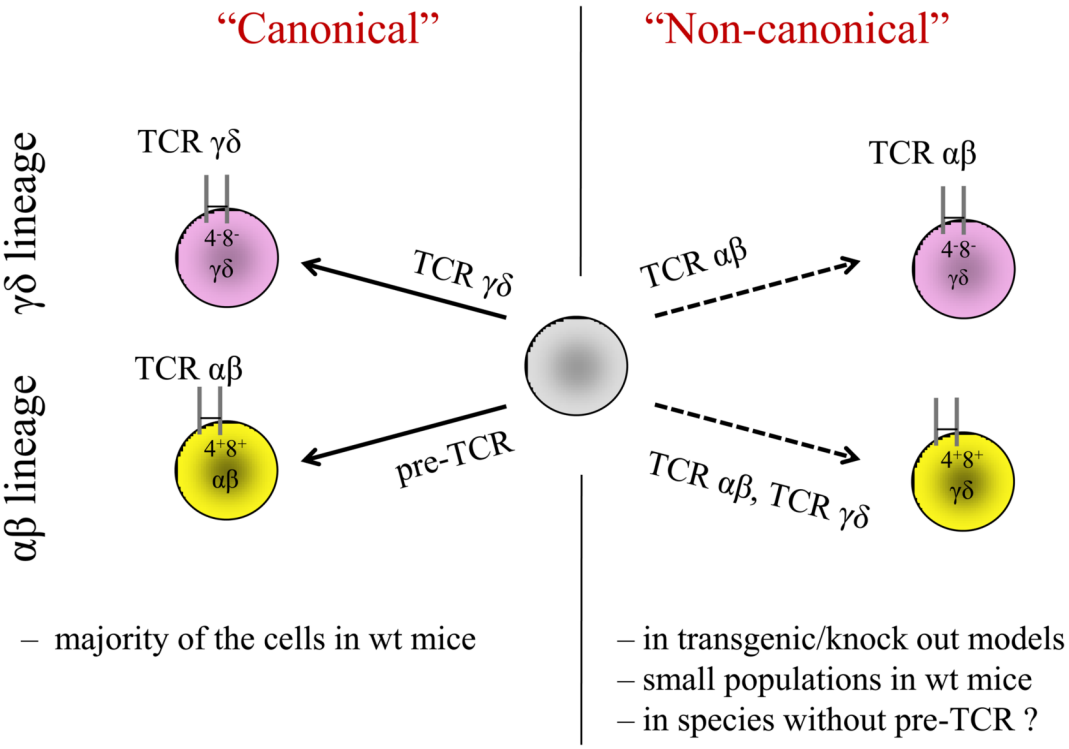

Figure 1. Lineages and TCR expression

In a wt mouse the majority of αβ lineage cells are initially driven by the pre-TCR which in case of a productive *Tcra* rearrangement is later replaced by the TCRαβ at the DP stage, whereas γδ lineage differentiation correlates well with TCRγδ expression. However, small populations in wt mice that are exaggerated in various knock-out and transgenic models do not follow these rules. Early TCRαβ expression can lead to the development of γδ lineage-like cells which avoid progression through the DP stage, whereas both TCRαβ and TCRγδ expression can support progression to the DP stage and thus αβ lineage differentiation in the absence of a pre-TCR. We speculate that these 'non-canonical' pathways might in fact be mainstream in the species that lack a pre-TCR.

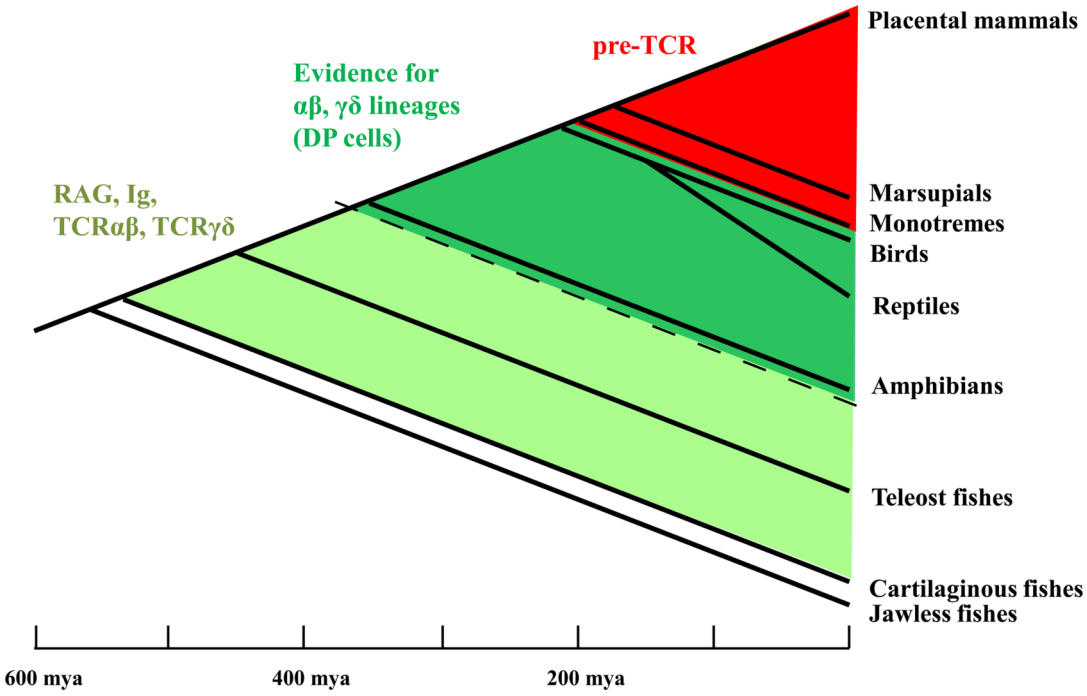

Figure 2. Evolution of TCRs and lineages

The four TCR chains are present in all vertebrates that undergo Rag-dependent rearrangement. It is not clear when the molecular programs corresponding to αβ and γδ lineages were always present, but the DP stage can be found in *Xenopus*, suggesting that certain features of the αβ lineage existed before the split between amphibians and reptiles. pTα homologs, however, can be found only in mammals, suggesting that αβ lineage differentiation initially did not rely on pre-TCR signaling. (mya – million years ago)

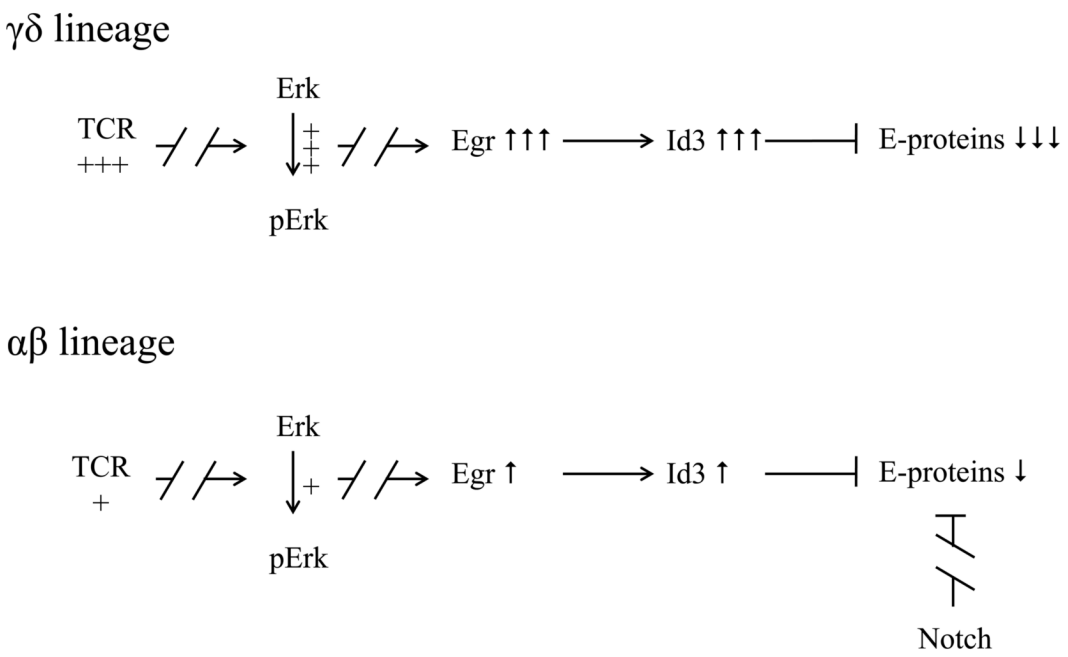

Figure 3. Possible role of E-protein activity inhibition in αβ versus γδ lineage choice

Strong TCR signals lead to potent inhibition of E-protein activity through strong induction of Id3, possibly via the Erk-Egr1 axis, which favors γδ lineage differentiation. Weaker TCR signals lead to weaker Id3 induction which in turn results in less profound inhibition of E-protein function and commitment to the αβ lineage. The dependence of αβ lineage development on strong Notch signaling might in this scenario be explained by the capability of Notch to further inhibit E-proteins (in Id3-dependent or independent manner).

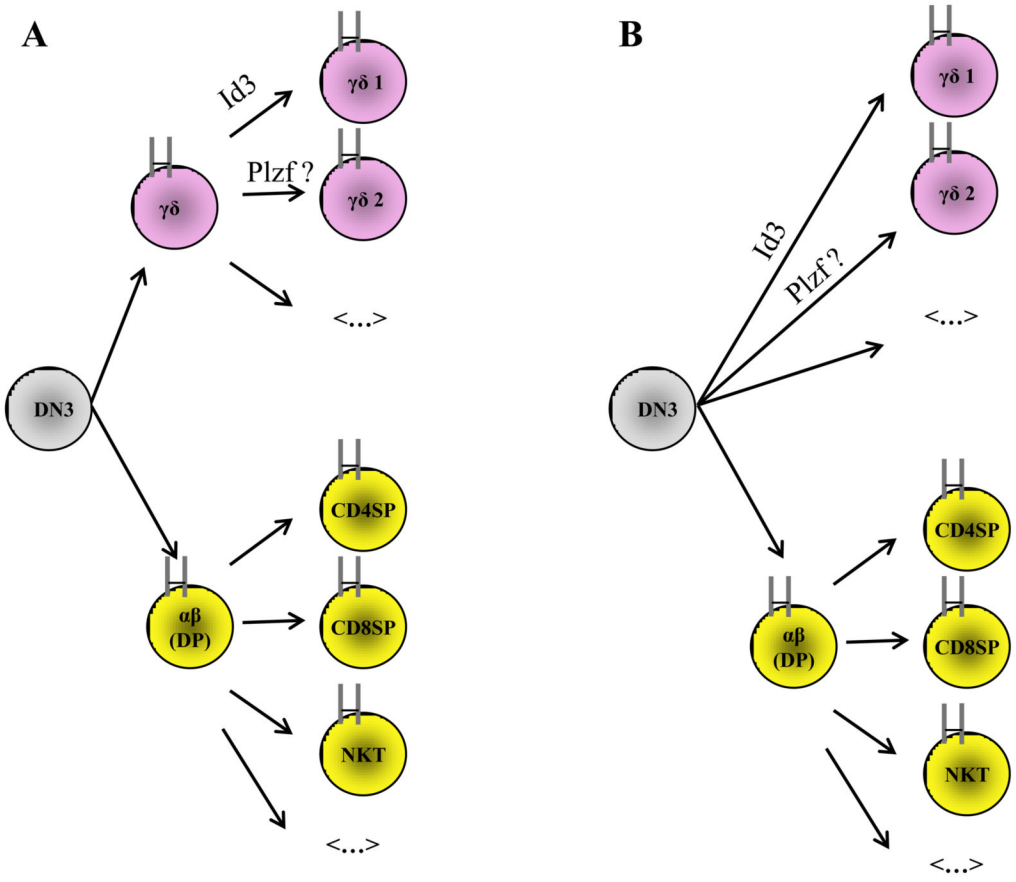

Figure 4. Two models of the lineage split at the first TCR-dependent checkpoint

A. TCR signaling leads to the execution of αβ or γδ lineage-specific molecular programs. At a later developmental stage sublineage-specific programs are initiated. **B.** TCR signaling, possibly in cooperation with other pathways, leads to the lineage split between the αβ lineage and several independent γδ lineages which do not share a common γδ molecular program.