T-cell development: **Is Notch a key player in lineage decisions?**

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At two consecutive 'checkpoints' in their development, T cells have to be rescued from programmed cell death and to choose between distinct lineage fates; recent results show that the Notch transmembrane receptor can significantly influence T-cell development at both of these points.

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As lymphocytes develop from pluripotent stem cells, in order to survive and mature they need to productively rearrange their antigen-receptor genes, which are present in pieces in the germ-line genome. Several 'checkpoints' have been identified at which developing T and B lymphocytes have to make a particular type of receptor in order to proceed to the next developmental stage. In the case of T cells, not only whether they will survive but also which particular lineage they will follow is determined at these checkpoints.

T-cell receptors come in two main kinds, αβ and γδ, both heterodimers; αβ and γδ T cells have distinct properties, however, so that the two lineages can be defined independently of the particular type of receptor expressed (see below). Early in the T-cell lineage, γ, δ and β T-cell receptor genes are rearranged (Fig. 1). If the γ and δ genes are rearranged productively — the component parts being joined in the correct reading frame — then the cells express a γδ T-cell receptor at the surface and subsequently follow the γδ lineage. If the γδ genes are not both rearranged productively, then the cells can go on and attempt to enter the αβ lineage. This is consistent with the finding that there is selection against productive γ and δ gene rearrangements in αβ lineage cells [1–3]

It appears that the decision to become a γδ T cell is not strictly determined by the type of receptor — either γδ or αβ — that is expressed, but rather by the timing of receptor expression. Thus, if an αβ T-cell receptor is expressed abnormally early in transgenic mice, then cells are formed with properties characteristic of being on the γδ lineage — for example, they crucially depend on the expression of the cytokine receptor γ chain [4] — but that nevertheless

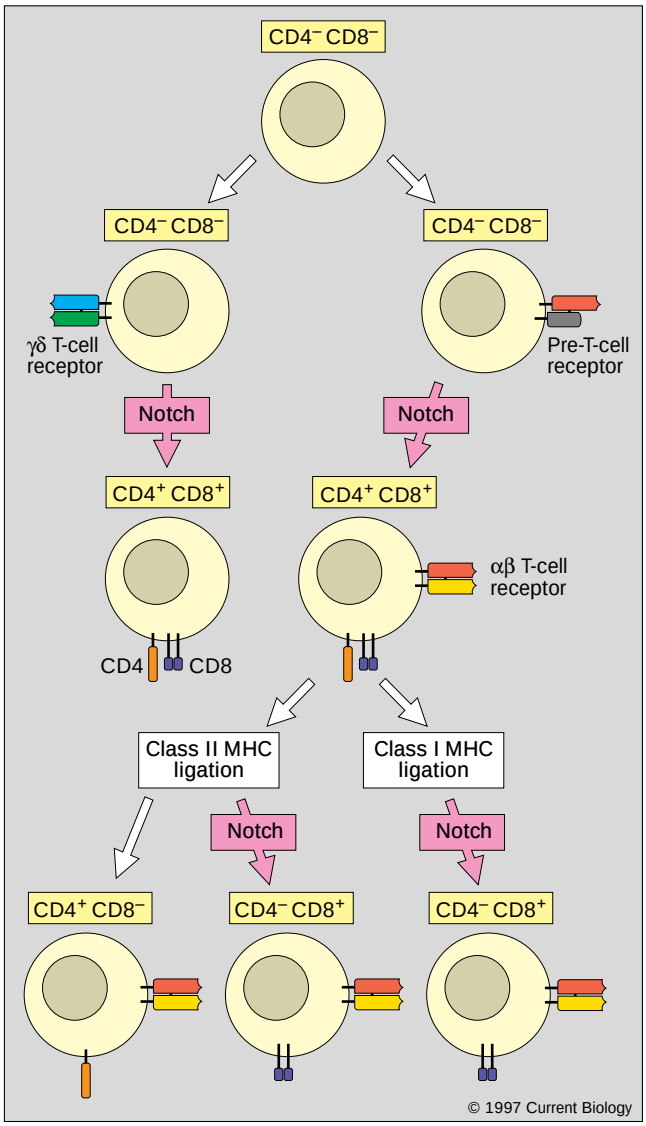

Figure 1

Early in T-cell development, the γ, δ and β chain T-cell receptor genes are rearranged, but the α chain gene remains unrearranged. Cells that can make a γδ T-cell receptor become γδ lineage cells; recent results show that Notch can induce expression of CD4 and CD8 in these cells, though most may consequently turn off cell-surface γδ T-cell receptor expression. Cells that cannot make a γδ T-cell receptor, but that productively rearrange a β-chain gene, express the 'pre-T-cell receptor' – a heterodimer of a β chain and a pre-T-cell receptor α chain. These cells expand, rearrange their α chain locus and become $CD4^+$ $CD8^+$ αβ-expressing cells; Notch supports the development of these cells. Immature $CD4^+CD8^+$ $\alpha\beta^+$ cells can be rescued from programmed cell death if their receptor binds to a class I or class II MHC molecule. Ligation of class I MHC molecules results in the formation of mature $CD4^-$ $CD8^+$ cells, and this pathway is promoted by Notch. Ligation of class II MHC molecules results in the formation of $CD4^+$ $CD8^-$ cells. Some $CD4^-$ $CD8^+$ cells with class II MHC specific T-cell receptors may result from the influence of an active Notch receptor.

express αβ, rather than γδ, T-cell receptors [5]. Early expression of either γδ or αβ T-cell receptor transgenes does not, however, entirely block the development of αβ T cells, indicating that neither the quality of the T-cell receptor nor the timing of T-cell receptor expression are the only factors determining commitment to the αβ lineage [5,6].

Cells that enter the αβ lineage first express 'pre-T-cell receptors', heterodimers of the T-cell receptor β chain and the 'pre-T-cell receptor' α chain (Fig. 1). Expression of this receptor by cells with a productively-rearranged β- chain gene rescues them from programmed cell death, terminates further rearrangement of the β-, δ- and probably also γ-chain genes, and induces a strong wave of proliferation in the selected cells that eventually express CD4 and CD8 cell-surface molecules [7]. CD4 and CD8 are believed to be markers of the αβ lineage; mature αβ T cells express either CD4 or CD8, which act as coreceptors in cells specific for peptide antigens bound to, respectively, class II or class I major histocompatibility complex (MHC) molecules. Mutant mice that lack the pre-T-cell receptor α chain can, however, still generate $CD4^+CD8^+$ cells in several ways, and some of these can express *bone fide* γδ T-cell receptors; again, therefore, the correlation between receptor type and cell phenotype is good, but not perfect [7].

A second checkpoint in the development of αβ T cells depends on the binding of the T-cell receptor to thymic MHC molecules, which can rescue immature αβ T cells from programmed cell death (Fig. 1). This rescue is again associated with lineage commitment: depending on whether the T-cell receptor is specific for class I or class II MHC molecules, the rescued cells will have the $CD4^-CD8^+$ cytolytic or $CD4^+CD8^-$ helper T-cell phenotype [8]. Even here, however, there is no perfect correlation between T-cell receptor specificity and lineage commitment, because $CD4^-CD8^+$ cytolytic T cells can be selected that express a T-cell receptor specific for class II MHC molecules [9].

At both these checkpoints in T-cell development, the molecular mechanisms specifying cell survival and, especially, lineage commitment have been largely unknown. Two recent papers [10,11] have now reported evidence implicating Notch as a key player in the lineage decisions at both of these checkpoints. Notch was originally identified in the fruitfly *Drosophila*, where, in ectodermal precursor cells, activated Notch inhibits neural differentiation so that the cells follow the alternative, epidermal fate. It appears that neighbouring precursors expressing slightly different levels of Notch and its ligand can influence each other in such a way that an initially small difference becomes exaggerated, and one cell comes to express only the receptor, and differentiates into an epidermal cell, and the other comes to express only the ligand, and differentiates into a neuron. This is thought to explain why, in mosaic fruitflies, heterozygous $Notch^{+/-}$ precursors — which presumably start off expressing a lower than normal level of Notch — adopt mostly the neural fate when next to wild-type precursors, but both neural and epidermal fates when developing in the absence of wild-type cells [12]. Notch homologs have been implicated in a number of different developing systems in various species. In hemopoietic cells of mammals, for example, Notch can inhibit differentiation into myeloid cells and thereby increase cell numbers of less differentiated hemopoietic precursors [13].

The two new studies [10,11] have investigated the role of Notch in lymphocyte — particularly T-cell — differentiation. Washburn *et al.* [10] studied lymphocyte development in mice with chimeric hematopoietic systems, in which some cells are wild-type and other heterozygous for a *Notch* mutation. They found that, in such chimeras, the $Notch^{+/-}$ precursors cells produced the same number of B cells, slightly fewer γδ T cells, but significantly fewer αβ T cells than were produced by the wild-type precursors. In contrast, in control chimeras where all the cells are $Notch^{+/-}$, the $Notch^{+/-}$ precursors produced the same ratio of αβ T cells to B cells as wild-type mice. From these observations, it was concluded that, in the presence of wild-type cells, cells expressing relatively low Notch receptor levels tend to favour the γδ over the αβ lineage. One caveat about these experiments is that data on the αβ to γδ lineage ratio were only presented for the wild-type–$Notch^{+/-}$ chimeras, and not for the control $Notch^{+/-}$–$Notch^{+/-}$ chimeras [10].

The caveat notwithstanding, additional data suggest that Notch does act to promote differentiation to the αβ T-cell lineage. An activated form of Notch was expressed from a transgene in mice that were relatively deficient in αβ lineage cells, either because they expressed γ and δ chain transgenes or because they lacked the β chain as a result of a mutation. In these mice, it was found that expression of activated Notch led to an increase in the proportion of $CD4^+CD8^+$ cells with productive γδ gene rearrangements [10]. In the β chain mutant mice, it was actually not clear whether there was an absolute increase in the number of $CD4^+CD8^+$ cells with or without productive γδ gene rearrangements; it did appear, however, that within the $CD4^+CD8^+$ population there had at some point been selection for cells with in-frame γδ gene rearrangements (although these cells no longer expressed γδ T-cell receptors at the cell surface). Gene rearrangement was clearly relevant to the observed effect of the activated Notch, as it did not affect development in rearrangement-deficient, *rag* mutant mice**.** It was concluded from these experiments that activated Notch could force γδ T-cell receptor expressing cells into the $CD4^+8^+$ αβ lineage, so that the cells adopt an αβ phenotype, even though they are carrying productively rearranged γ- and δ-chain genes, but not productively rearranged α- or β-chain genes.

In the other paper, Robey *et al.* [11] studied the effect of expressing activated Notch on the second checkpoint in T-cell development: the point at which cells choose whether to develop into $CD4^-CD8^+$ or $CD4^+CD8^-$ cells. They found that expression of activated Notch increased the production of $CD4^-CD8^+$ cells and decreased the production of $CD4^+CD8^-$ cells. The activated Notch also caused an increase in the proportion of $CD4^-CD8^+$ cells in class I MHC-deficient mutant mice, but not in mice deficient in expression of both class I and class II MHC molecules [11]. Robey *et al.* [11] concluded that Notch activity forces cells into the $CD4^-CD8^+$ lineage, and away from the $CD4^+CD8^-$ lineage.

Although the interpretation of these very interesting observations [10,11] is still open to argument, it is clear that activated Notch can have a profound effect on T-cell development. What is less clear is the mechanism by which Notch exerts its effects: is it really the lineage decision that is made by Notch? This question is difficult to answer, because it is conceivable that Notch confers selective survival and/or cell proliferation on cells that have already made a lineage decision, but that are not fully differentiated. This would explain the increase in the proportion of 'unorthodox' cells — like the $CD4^-CD8^+$ cells expressing class II MHC-specific αβ T-cell receptors, or the $CD4^+CD8^+$ cells with productively rearranged γδ T-cell receptor genes.

It is more difficult to explain the apparent deficit in some T-cell subsets seen in certain situations. This may reflect a competition that favours cells expressing higher levels of Notch. In the case of the wild-type–*Notch*$^{+/-}$ chimeras, for instance, one could argue that the wild-type αβ lineage precursors outcompete the *Notch*$^{+/-}$ precursors, and that this is the only reason why they contribute more to the αβ lineage. In the case of the $CD4^-CD8^+$ T cells, one could argue that these cells, when expressing higher Notch levels, outcompete $CD4^+CD8^-$ cells that express lower Notch levels soon after the lineage decision has been made. This would be consistent with the notion that Notch favours survival or expansion of some hemopoietic cells. [13].

Thus, in a strict sense, the new data do not directly show that Notch makes lineage decisions during lymphocyte development, although they are perfectly consistent with such a notion The final word is not in on this issue, but these experiments have certainly added another level of complexity to our appreciation of lymphocyte development and lymphocyte lineage decision.

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