T-cell lineage commitment revisited

Years of controversy about the lineage relationship between αβ and γδ T cells may at last have been resolved: it now appears that most T cells derive from an identical T-lineage committed precursor.

Hematopoietic stem cells formed in the fetal liver and the bone marrow eventually follow one of several fates. Those that colonize the thymus eventually commit to become T lymphocytes and initiate developmental events that ultimately transform them into mature effector T cells equipped to play important roles in immune responses [1]. Maturing T cells undergo DNA rearrangements that shuffle variable (V) and constant (C) region gene segments to generate the genes coding for the T-cell antigen receptor. Surface-expressed T-cell receptors endow T lymphocytes with recognition properties allowing discrimination between self and non-self. At the population level, T-cell receptor diversity is enormous, but individual T cells express only one of these many variants consisting of an α and a β chain or, in a mutually exclusive fashion, of a γ and a δ chain. For proper assembly and function, the T-cell receptor requires further association with a set of signaling proteins collectively referred to as the CD3 complex [2].

In ontogeny, γδ gene rearrangement and expression precedes the appearance of αβ T cells. The first cells to don a T-cell receptor appear at about day 14 of mouse gestation and bear Vγ3 and Vδ1 chains. Shortly thereafter, T cells expressing Vγ4, also paired with Vδ1, can be detected. These early V genes are expressed only in the fetal period and are undetectable in the thymus after birth [3]. Two features endow early γδ T cells with rather interesting properties not shared by most other T cells. First, their T-cell receptors exhibit no junctional diversity, creating essentially monoclonal cell populations. Second, these cells demonstrate a remarkable tissue distribution, with Vγ3 T cells populating the epidermis and Vγ4 T cells homing to the reproductive epithelium.

Considered as a whole, this information suggested that early γδ T-cell populations may perform tissue-specific functions [4]. Evidence obtained in our and other laboratories previously established that these two initial T-cell waves required for their production both a stem cell population and a thymic microenvironment of fetal origin [5,6]. Furthermore, the developmental potential of this fetal stem cell population appears limited to early γδ T cells [7]. In contrast, cells expressing other γδ or αβ T-cell receptors derive from a different stem cell population, also appearing during the fetal period but persisting well into adulthood (Fig. 1). These T cells become detectable by day 16–17 of gestation. From then on, cells having αβ T-cell receptors become the predominant T-lymphocyte population in the thymus.

What is the lineage relationship between these late-appearing γδ and αβ T cells? In 1987, Allison, Pardoll and colleagues [8,9] proposed a sequential model for lineage commitment based on the observation that γδ T cells appear before αβ T cells. According to this model, if T-cell receptor γ and δ genes cannot productively rearrange, then T-cell receptor α and β genes attempt rearrangements. This scheme predicts that αβ T cells originate from failed γδ T cells. Alternative models proposed instead that γδ and αβ T cells have separate origins and predicted that rearrangement of T-cell receptor α and δ genes were mutually exclusive events. Several groups initiated molecular genetic experiments to test these models. The experiments, however, were complicated by the location of all δ T-cell receptor gene segments within the T-cell receptor α locus (Fig. 2).

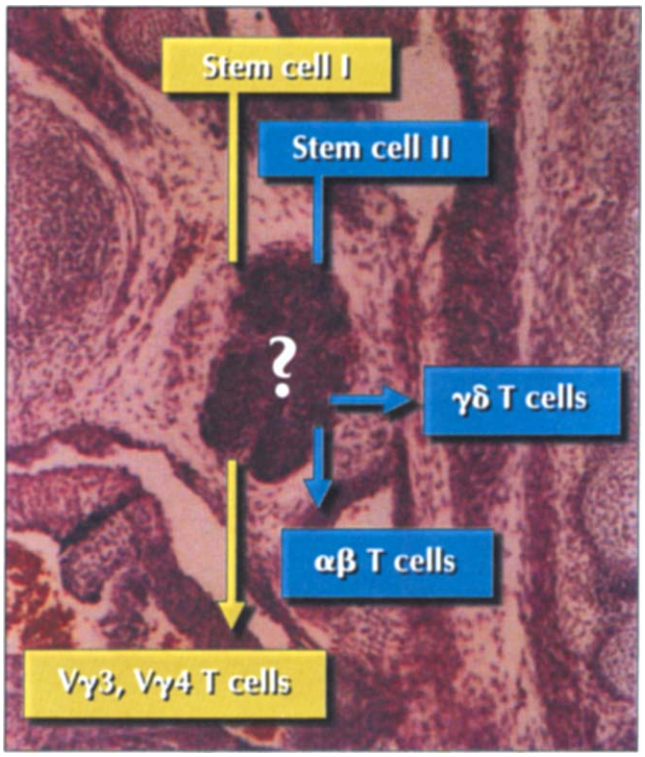

Fig. 1. Developmental fate of precursor T-cell populations in the thymus. An early fetal stem cell population can differentiate within a fetal thymus to generate T-cell receptor Vγ3- and Vγ4-bearing monoclonal T-cell populations residing in the epidermis and reproductive epithelia, respectively. A different stem cell population can reconstitute all other αβ and γδ T cells observed in the neonate and adult. Events leading to lineage commitment in this later-appearing precursor cell population have remained controversial (see text).

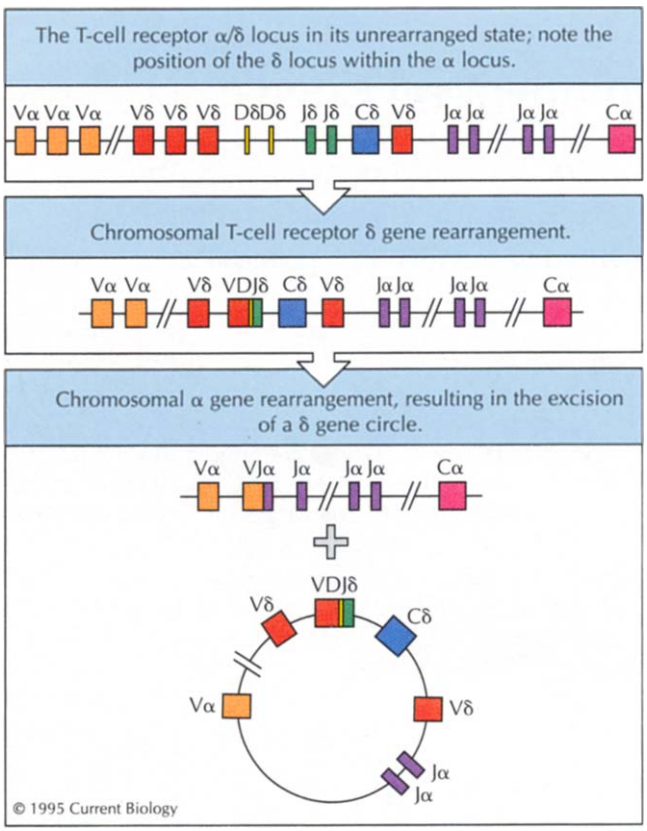

Fig. 2. Schematic depiction of T-cell receptor δ gene rearrangement, followed by T-cell receptor α gene rearrangement and concomitant excision of a T-cell receptor δ gene circle. Adapted from [14].

Chromosomal T-cell receptor α gene rearrangement thus results in the excision of T-cell receptor δ gene segments as circular pieces of DNA. In principle, the thymus should be a rich source of such DNA circles, and indeed three groups have successfully created T-cell receptor δ circle DNA libraries, but subsequent analyses of these libraries generated conflicting data [10–12]. The reasons for the observed discrepancies remain unresolved, but may relate to differences in procedures used to purify DNA circles or an inherent lack of quantitative representation in DNA circle libraries. These difficulties notwithstanding, a different set of experiments provided strong evidence against the sequential-rearrangement model. Several studies identified DNA elements mediating transcriptional repression of T-cell receptor γ genes in αβ but not γδ T cells. The ability to commit to a particular T-cell lineage may thus depend on the propensity of different precursor cell populations to produce factors silencing the T-cell receptor γ locus. In 1990, following reports of additional studies in transgenic mice, it became commonly accepted that differentiation along the αβ and γδ T cell lineages was independently regulated [13].

Now, however, Dudley *et al.* [14] and Livak *et al.* [15] challenge this view following quantitative assessments of T-cell receptor δ gene rearrangement in mature αβ T cells. These cells retain rearranged T-cell receptor δ sequences within a germ-line configured α gene or as part of excised DNA circles, as demonstrated in these recent publications as well as in a previous study [12,14,15]. This finding is in line with observations that large numbers of αβ T cells never initiate active division, an event that would inevitably result in the lost of extrachromosomal DNA circles. Both groups, then, could analyze the frequency of productive T-cell receptor δ gene rearrangement in αβ T cells. If the gene rearrangements are random, in a third of cases they should preserve the translational reading frame leading to the synthesis of a functional T-cell receptor protein. Such a frequency of functional rearrangements would be expected if lineage determination occurs independently of T-cell receptor gene rearrangements.

However, a sampling of several hundred T-cell receptor δ gene rearrangements revealed a significantly reduced frequency of in-frame δ rearrangements in mature αβ T cells [14,15]. This depletion of productive δ gene rearrangements in αβ T cells supports the hypothesis that a proportion of cells with productive T-cell receptor δ chains are removed from the αβ T-cell precursor pool and may eventually become γδ T cells. Under conditions in which T-cell receptor δ chains do not reach the cell surface, the frequency of in-frame T-cell receptor δ gene rearrangement should rise to that expected for a random process.

This prediction was successfully tested by Dudley *et al.* [14] using 'knock-out' mice that had normal T-cell receptor gene rearrangement but were unable to synthesize T-cell receptor δ chains. As the observed depletion in normal mice was partial rather than total, at least one other molecule must be involved in the removal of a proportion of cells that can express δ chains from the precursor αβ T-cell population. The most likely candidate was the T-cell receptor γ chain. An analysis of T-cell receptor γ gene rearrangement in αβ T cells by Dudley *et al.* [14] revealed a depletion of productive rearrangements similar to that noted at the T-cell receptor δ locus. This additional finding provides further evidence that T-cell precursors first try to rearrange T-cell receptor γ and δ genes in an attempt to become γδ T cells. Cells failing to express a γδ T-cell receptor then try to become αβ T cells (Fig. 3).

Clearly, conditions must limit the capacity of precursor cells to develop into γδ T cells, as most become αβ T cells. Livak *et al.* [15] propose that lineage-commitment decisions result from a competition between signals generated by expressed γδ T-cell receptors and some ill-defined αβ T-cell commitment events. The contemporaneous rearrangement of T-cell receptor β, γ and δ chain genes provides one level at which such competition could be waged. In the absence of a T-cell receptor α chain, the recently described pre-T-cell receptor complex is a likely candidate for being a source of signals that direct precursors to differentiate into αβ T cells [16]. The pre-T-cell receptor is composed of a productively rearranged T-cell receptor β chain gene and a newly

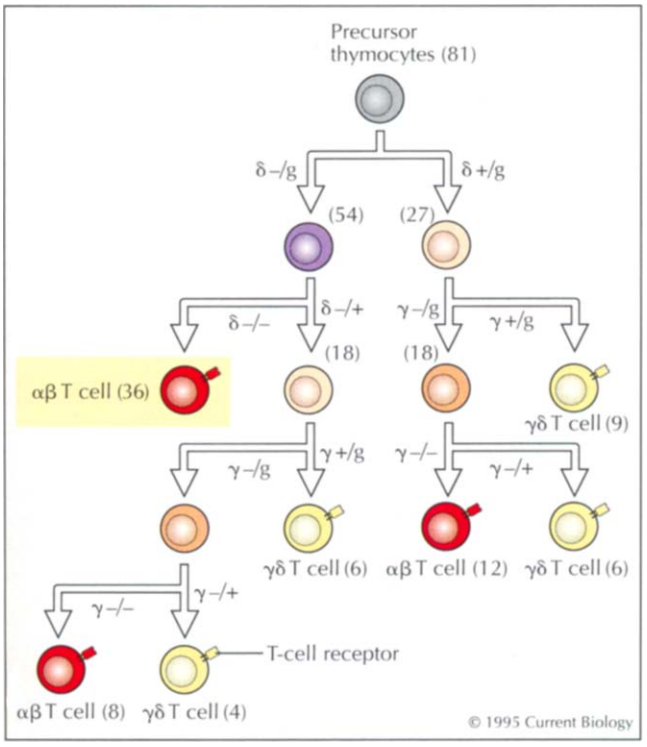

Fig. 3. The sequential-rearrangement model for T-cell development, circa 1995. As shown, 56 αβ T cells (12 + 8 + 36) would derive from every 81 cells following this pathway. This would require that they have an out-of-frame rearrangement of either the δ or γ genes. The denoted αβ subset (highlighted by a yellow box) is able to rearrange randomly at the γ locus, as there is no possibility of making a γδ T-cell receptor. Allele states are indicated by + (in-frame), – (out-of-frame) and g (germ-line). Numbers in parentheses indicate the number of cells that would be expected to constitute a given subset, from an original 81 T-cell precursors. Adapted from [14].

cloned 33 kDa glycoprotein known as pre-T-cell receptor α (pTα). Current evidence suggests that the pre-T-cell receptor serves to signal the productive expression of a T-cell receptor β chain, thereby ensuring clonotypic expression and allowing further maturation [17].

The role that signaling through these different receptors plays in lineage commitment remains to be dissected. At a minimum, we can infer from recent experiments with transgenic mice that signaling by T-cell receptor γδ or pTαβ complexes can generate similar maturation signals in precursor cells [18]. However, the detection of productive T-cell receptor β rearrangement in a large proportion of mature γδ T cells by Dudley *et al.* [14] argues against a strict competition model. Instead, precursor T cells would be required to remain uncommitted for some time following productive T-cell receptor β gene rearrangement. This condition could be met if signaling through the pre-T-cell receptor occurs only following ligand recognition. Alternatively, as proposed by Dudley *et al.* [14] lineage commitment may depend on the time of activation of T-cell receptor α gene rearrangement. The two models are not, of course, mutually exclusive.

The new data fulfill most predictions of the sequential-rearrangement model for T-cell lineage commitment. Revision of the Allison and Pardoll model is necessary to account for the early developmental onset of T-cell receptor β gene rearrangement. On the other hand, the new findings are inconsistent with T-cell lineage models based on the mutually exclusive rearrangement of T-cell receptor α and δ genes, or transcriptional regulation of the T-cell receptor γ locus. Indeed, recent observations indicate that transcriptional silencing may play an important role in maintaining, rather than establishing, lineage commitment [19]. A renewed interest in lineage commitment will probably surface from the developmental issues raised by the new evidence. These considerations should occupy immunologists for some time.

Acknowledgements: We thank Ann Feeney and Stephen Hedrick for critical reading of the manuscript and for helpful discussions.

References

1. Rodewald H-R: **Pathways from hematopoietic stem cells to thymocytes.** *Curr Opin Immunol* 1995, **7**:176–187.
2. Weiss A, Littman DR: **Signal transduction by lymphocyte antigen receptors.** *Cell* 1994, **76**:263–274.
3. Allison JP, Havran WL: **The immunobiology of T cells with invariant γδ antigen receptors.** *Annu Rev Immunol* 1991, **9**:679–705.
4. Havran WL, Boismenu R: **Activation and function of γδ T cells.** *Curr Opin Immunol* 1994, **6**:442–446.
5. Havran WL, Carbone A, Allison JP: **Murine T cells with invariant γδ antigen receptors: origin, repertoire, and specificity.** *Semin Immunol* 1991, **3**:89–97.
6. Ikuta K, Kina T, MacNeil I, Uchida N, Peault B, Chien Y-H, Weissman IL: **A developmental switch in thymic lymphocyte maturation potential occurs at the level of hematopoietic stem cells.** *Cell* 1990, **62**:863–874.
7. Heyborne K, Fu Y-X, Kalataradi H, Reardon C, Roark C, Eyster C, Vollmer M, Born W, O'Brien R: **Evidence that murine Vγ5 and Vγ6 γδ-T-cell receptor$^+$ lymphocytes are derived from a common distinct lineage.** *J Immunol* 1993, **151**:4523–4527.
8. Allison JP, Lanier LL: **The T-cell antigen receptor gamma gene: rearrangement and cell lineages.** *Immunol Today* 1987, **8**:293–296.
9. Pardoll DM, Fowlkes BJ, Bluestone JA, Kruisbeek A, Maloy WL, Coligan JE, Schwartz RH: **Differential expression of two distinct T-cell receptors during thymocyte development.** *Nature* 1987, **326**:79–81.
10. Okazaki K, Sakano H: **Thymocyte circular DNA excised from T cell receptor α–δ gene complex.** *EMBO J* 1988, **7**:1669–1674.
11. Winoto A, Baltimore D: **Separate lineage of T cells expressing the αβ and γδ receptors.** *Nature* 1989, **338**:430–432.
12. Takeshita S, Toda M, Yamagishi H: **Excision products of the T cell receptor gene support a progressive rearrangement model of the α/δ locus.** *EMBO J* 1989, **8**:3261–3270.
13. Haas W, Tonegawa S: **Development and selection of γδ T cells.** *Curr Opin Immunol* 1992, **4**:147–155.
14. Dudley EC, Girardi M, Owen MJ, Hayday AC: **αβ and γδ T cells can share a late common precursor.** *Curr Biol* 1995, **5**:659–669.
15. Livak F, Petrie HT, Crispe IN, Schatz DG: **In frame TCR δ gene rearrangements play a critical role in the αβ/γδ T cell lineage decision.** *Immunity* 1995, in press.
16. Roth PE, DeFranco AL: **Intrinsic checkpoints for lineage progression.** *Curr Biol* 1995, **5**:349–352.
17. Dudley EC, Petrie HT, Shah LM, Owen MJ, Hayday AC: **T cell receptor beta chain gene rearrangement and selection during thymocyte development in adult mice.** *Immunity* 1994, **1**:83–93.
18. Kersh Gj, Hooshmand FF, Hedrick SM: **Efficient maturation of αβ lineage thymocytes to the CD4$^+$CD8$^+$ stage in the absence of TCR-β rearrangement.** *J Immunol* 1995, **154**:5706–5714.
19. Sim G-K, Olsson C, Augustin A: **Commitment and maturation of the αβ and γδ T cell lineages.** *J Immunol* 1995, **154**:5821–5831.

Richard Boismenu and Wendy L. Havran, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California 92037, USA.