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Untangling the T branch of the Hematopoiesis Tree

Anthony W. Chi, J. Jeremiah Bell, Daniel A. Zlotoff, and Avinash Bhandoola

Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA

Summary

T cells develop in the thymus. Previous work suggested an early separation of lymphoid from myeloerythroid lineages during hematopoiesis, and hypothesized the thymus was settled exclusively by lymphoid-restricted hematopoietic progenitors. Recent data have instead established the existence of lymphoid-myeloid progenitors, which possess lymphoid and myeloid lineage potentials but lack erythroid potential. Myeloid and lymphoid potentials are present at the clonal level in early thymic progenitors, confirming that progenitors settling the thymus include lymphoid-myeloid progenitors. These results revise our view of the T lineage branch of hematopoiesis, and focus attention on the generation, circulation, and homing of lymphoid-myeloid progenitors to the thymus.

Introduction

T cells are unique among blood cells, as they require a dedicated organ for their development, the thymus. Progenitors in the thymus lack self-renewing capacity, and so T lymphopoiesis requires periodic import of hematopoietic progenitors from the blood. These progenitors must leave the bone marrow (BM), traffic through the circulation, and settle the thymus before developing into mature T cells.

In this review, we describe differences between past and revised models of T lineage development in adult mice. We discuss developmental potentials of early thymic progenitors (ETPs), as they represent the most immature thymocytes identified so far. We describe extrathymic progenitors that are candidate sources of T cell progenitors, with an emphasis on thymus homing ability. We summarize recent advances in identifying thymus-settling progenitors as the link between BM and the thymus in T lymphopoiesis.

Two models of T lineage development

All blood cell types originate from multipotent, self-renewing hematopoietic stem cells (HSCs), via non-renewing multipotent progenitors (MPPs), but developmental steps downstream of MPPs are less well understood (Figure 1). One model of hematopoiesis proposed a strict separation of lymphoid and myeloerythroid lineages [1]. This idea was supported by the identification of common myeloid progenitors (CMPs) and common lymphoid progenitors (CLPs) in BM [1]. Implicit in this model was the notion that the thymus is settled by lymphoid-restricted progenitors with developmental potential for T, B, and NK lineages. However, current data suggest a revised model in which erythroid potential is lost but myeloid potential is retained in progenitors of lymphocytes (Figure 1). This is supported

Correspondence: Avinash Bhandoola; E-mail: bhandoola@mail.med.upenn.edu.

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by strong evidence for the existence of progenitors that have lost erythroid potential but retain lymphoid as well as myeloid potentials (LMPs) [2–6]. In the revised model, the thymus is settled by such lymphoid-myeloid progenitors. One job of the thymus is therefore to restrict such multipotential progenitors to the T cell lineage.

Early thymic progenitors (ETPs)

The precise identity of hematopoietic progenitors that settle the thymus is difficult to know with certainty, due to the fact that many extrathymic progenitors possess T lineage potential and only a few cells are needed to settle the thymus for robust thymopoiesis [7]. Consequently, identifying and characterizing the most immature thymocytes with T lineage potential in the thymus has been a focus of many laboratories, as these cells might represent the thymus-settling progenitors or their immediate progeny. Early studies demonstrated that cells with a $CD4^{-/low}CD3^{-}CD8^{-}CD25^{-}CD44^{+}$ surface phenotype (termed double-negative 1 or DN1) were the least mature thymocytes [7]. Further examination of this population showed that efficient T lineage progenitor activity resides in the Kit^{+} subset, which lacks surface markers associated with terminally differentiated blood lineages. These efficient T cell progenitors are termed early thymic progenitors (ETPs, minimally defined as $Lin^{-}CD25^{-}Kit^{+}$) and constitute approximately 0.01% of the young adult mouse thymus [7–11]. ETPs have been referred to as “canonical” T cell progenitors because of their tremendous efficiency in generating downstream DN2 ($CD25^{+}Kit^{+}$), DN3 ($CD25^{+}Kit^{-}$), and double-positive ($CD4^{+}CD8^{+}$) T lineage progeny [10,11](Figure 2). ETPs are themselves heterogeneous for expression of several surface markers, including CD24, Flt3, and CCR9, suggesting the existence of developmental transitions even within this rare progenitor population [11–13]. $Flt3^{+}$ ETPs represent a small subset of ETPs, and overlap with the EGFP-brightest subset of ETPs in CCR9-EGFP reporter mice [12–14]. This subset of ETPs appears more primitive than others that lack expression of the Flt3 receptor [12].

The ability to constrain alternative lineage cell fates by the thymus

The majority of ETPs retain developmental potentials for NK and DC lineages at the clonal level [13–20]. Rare ETPs within the $Flt3^{+}CCR9^{+}$ subset can also give rise to B cells; however, the vast majority of ETPs lack B potential [11–14,20]. Most recently, it has been shown that ETPs possess myeloid potential [19,20] in clonal assays [17•,18•]. Downstream of ETPs, some DN2 cells can still give rise to NK, DC and myeloid cells, but most are committed to the T cell lineage [15–18•]. The T cell fate is further consolidated at the DN3 stage, where developmental potential for alternative lineages is completely lost.

The presence of non-T lineage potentials in ETPs necessitates mechanisms within the thymus that promote the T cell fate while inhibiting development of alternative lineages. Recently Delta-like 4 was shown to be expressed in thymic epithelial cells and responsible for activating Notch1 signaling in incoming progenitors [21•,22•]. In DN3 thymocytes, Notch1 acts as a trophic factor, ensuring growth, proliferation and survival [23]. In ETPs, however, Notch1 signaling is likely required to restrict progenitors to the T cell fate, as withdrawal of Notch1 signals in ETPs leads to development of alternative lineages [6,24]. Mechanisms downstream of Notch1 signaling that direct lineage restriction are currently not well understood.

Multi-lineage Hematopoiesis in the Thymus

Despite evidence for active Notch1 signaling in ETPs [12,25], cells of alternative lineages are generated from ETPs in the thymus. Early work showed that intrathymic T progenitors could give rise to thymic dendritic cells (DC) [26]. Some natural killer (NK) cells in the periphery were found to express rearranged TCR γ genes; these rearrangements could not be detected in NK cells of athymic nude mice, suggesting some NK cells develop in the thymus [27,28]. The

thymus is also estimated to produce and export approximately 2×10^4 mature B cells daily [29]. Most recently, thymic granulocytes and macrophages have been shown to arise from ETPs, further establishing that thymic settling progenitors possess myeloid lineage potential among their non-T lineage potentials, and that this potential is realized in the thymic niche [17•,18•]. How some ETPs escape the T cell fate to instead generate NK and myeloid cells, and possibly cells of other lineages, awaits further investigation.

Multiple extrathymic progenitors with T lineage potential

Notch1 signaling is necessary for T lineage development [30]. Thus, progenitors with T lineage potential must be able to express Notch1 receptors and respond to Notch1 inductive signals. Several progenitors in adult mouse BM express Notch1 and possess T lineage potential, including HSCs. The most primitive hematopoietic progenitors reside within a rare subset that bears the “LSK” phenotype ($\text{Lin}^- \text{Sca-1}^+ \text{Kit}^+$). This LSK pool is heterogeneous and contains multipotent and self-renewing HSCs, non-self-renewing MPPs, and also early lymphoid progenitors (ELPs) that are lymphoid-specified since they express lymphoid-specific genes such as RAG1 and RAG2 [4,5]. ELPs are efficient lymphoid progenitors, but retain myeloid lineage potential [5]. They overlap extensively with a population of LSK progenitors expressing the highest levels of Flt3, which are named lymphoid-primed multipotential progenitors or LMPPs [4] – we use ELP and LMPP terms interchangeably in this review.

Several progenitors outside of the LSK pool also possess T lineage potential, including common lymphoid progenitors (CLPs) as well as early progenitors with lymphoid and myeloid developmental potential (EPLMs) that overlap with common lymphoid progenitors–2 (CLP-2) cells [1,31,32]. Krueger and von Boehmer have reported a T lineage-biased progenitor in the blood, which they termed circulating T cell progenitors (CTPs) [33]. An independent group earlier reported a rare population in BM, also named CTPs for committed T cell progenitors [34]. Both CTPs share a similar phenotype; a developmental relationship between these cells and phenotypically similar cells in spleen has been suggested [35]. Surface phenotypes of these progenitors have been recently reviewed [36] and are not repeated here. Most recently, a sub-population of BM cells of the $\text{Lin}^- \text{Sca-1}^+ \text{Kit}^-$ phenotype was shown to also possess T lineage potential [37,38].

Lymphoid-myeloid progenitors

As HSCs differentiate towards ELPs, their erythroid potential is lost [2,4,39•,40]. Consequently, ELPs are lymphoid-myeloid progenitors. Further downstream, ELPs give rise to CLPs. However, the “CLP” term appears at least in part a misnomer, because myeloid potential remains evident in some “CLPs” and possibly even further downstream in some CLP-2 cells [19,32,37,38,41]; which might indicate heterogeneity within populations previously considered to be lymphoid-restricted. Hence ELPs and a subset of CLPs, as well as additional progenitor cell types, may all represent lymphoid-myeloid progenitors (LMPs, Figure 2) in the BM.

Requirements for generation of lymphoid-myeloid progenitors

Precursor-successor relationships among progenitors downstream of HSCs are still being charted, but it seems clear that HSCs initially give rise to MPPs, which can differentiate into ELPs and downstream cells (Figure 2). What regulates the transition from MPPs to ELPs is not well understood; however, some critical requirements have been identified. Recent results suggest Wnt4 signaling may affect these developmental stages, via a non-canonical (β -catenin-independent) pathway [42]. In addition, Ikaros has been implicated in generating functional ELPs and CLPs, as these subsets are absent in Ikaros null mice. Flt3 expression within the LSK compartment depends on Ikaros [10,43•]. Perhaps consistent with this observation, Flt3

signaling is necessary for the generation of ELPs [44,49•]. E2A proteins are crucial for generation of ELPs as well. Similar to Flt3 signaling, E2A transcription factors also upregulate a range of lymphoid-associated genes, while suppressing nonlymphoid genes. In particular, CCR9 and Notch1 expressions in ELPs are controlled by E2A, highlighting its role in generating progenitors competent to home to the thymus and initiate T cell development [39•,45].

Selective homing of progenitors to the thymus

For a BM cell to become a successful T lineage progenitor, it must have the ability to migrate to the thymus. Most of the described progenitors with T cell potential are known to circulate [46]. The ability of progenitors to settle the thymus, on the other hand, is clearly selective and regulated. A number of chemokine receptors and adhesion molecules have been reported to regulate thymic settling, including CCR7, CCR9, PSGL1, and $\alpha 4$ and $\beta 2$ integrins [47–51]. Among them, the role of CCR9 for optimal homing of prethymic progenitors to the thymus has been repeatedly demonstrated [47–49•,51]. Whether additional molecules are implicated in thymic homing requires further study.

Identification of thymic homing progenitors

The identification of thymic homing molecules allows a molecular definition of thymic homing progenitors, because any such progenitors must express the necessary complement of molecules required for thymic homing. Interestingly, CCR9 is differentially expressed among the BM progenitors mentioned above. By using CCR9-GFP knock-in mice, Benz and Bluel first reported a group of CCR9⁺ LSK cells found in both BM and blood [13]. It was later confirmed that these were ELPs [48•,49•,52••].

Indeed, a high frequency of ELPs expresses CCR9, and these progenitors appear efficient in colonizing and repopulating the thymus from the circulation. In contrast, HSCs and MPPs lack CCR9 expression and do not home to the thymus effectively [48•,49•,53]. The most primitive subset of ETPs, the Flt3⁺ ETPs, also expresses CCR9 transcripts, and this subset of ETPs is phenotypically very similar to CCR9-expressing ELPs in the bone marrow [48•,52••]. Independent of Notch1 signaling, CCR9⁺ ETPs have been shown to be the first host progenitor cells appearing within a transplanted thymus [52••]. All these observations support the idea that CCR9-expressing ELPs are likely to represent one type of thymus settling progenitor. However, this is unlikely to represent the full story. In addition to CCR9⁺ ELPs, other progenitors including CLPs and perhaps CLP-2 cells may also home to thymus and contribute to T lymphopoiesis [31,32,37,38,51]. A contribution from these progenitors seems likely based on the expression of Notch1 as well as thymic homing molecules on these populations. It further remains possible that T lineage-committed CTPs also home to the thymus, where they may contribute to T lineage development via a non-canonical pathway that circumvents the ETP step [33,34]. All of these possibilities require further investigation.

Concluding remarks

T cell development spans multiple anatomic sites. Progenitors with T potential are generated in the BM and must migrate to the thymus. The classic hematopoietic tree dictated an early separation of lymphoid and myeloerythroid development and proposed that the thymus was settled exclusively by lymphoid-restricted progenitors. Perhaps predictably, things are more complicated. The multiple lineage potentials found in ETPs indicate at least some incoming progenitors must possess myeloid and lymphoid lineage potentials. In line with the revised model (Figure 1), the least mature human thymocyte progenitors also possess myeloid and lymphoid lineage potentials in clonal assays [54]. Downstream of ETPs, developmental potentials for alternative lineages are slowly constrained at least in part by Notch1 signals, and

finally lost at the DN3 stage. CCR9-expressing ELPs have recently been demonstrated as one possible type of thymus-settling progenitor that gives rise to ETPs. However, it seems plausible that other progenitors may contribute to T lymphopoiesis as well.

The presence of non-T lineage potentials in ETPs raises interesting questions. Is there a reason myeloid and lymphoid lineage potentials are “bundled” together in one group of thymus homing progenitors? Cells of several non-T hematopoietic lineages including dendritic cells, macrophages and granulocytes are present in the adult thymus, and some of these lineages are required for proper thymic function. It seems clear that at least some of these non-T lineage cells arise from ETPs. How are T lymphoid potential as well as alternative lineage potentials of ETPs realized in the thymus? Are some intrathymic niches protected from Notch signaling? Alternatively, might some ETPs express negative regulators of the Notch pathway? In addition to lymphoid-myeloid progenitors, other progenitor types may also migrate to the thymus to help generate non-T cell hematopoietic lineages; such additional progenitors remain to be better identified. Future studies will need to examine how these pathways of T versus accessory cell development in the thymus contribute to T cell development, and whether these pathways are perturbed in circumstances such as BM transplantation and lymphopenic diseases.

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Abbreviations

CCR9	chemokine (C-C motif) receptor 9
CLP	common lymphoid progenitor
CMP	common myeloid progenitor
CTP	circulating T cell progenitor, committed T cell progenitor
DC	dendritic cell
ELP	early lymphoid progenitor
EPLM	early progenitor with lymphoid and myeloid developmental potential
ETP	early thymic progenitor
HSC	hematopoietic stem cell
LMP	lymphoid-myeloid progenitor
LMPP	lymphoid-primed multipotent progenitor

MPP

multipotent progenitor

NK

natural killer cell

TSP

thymus-settling progenitor

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Classical Model

Revised Model

Figure 1. Past and revised models of hematopoiesis

The standard classical model (top) theorized that there is a strict separation of myeloerythroid and lymphoid development. This model proposes that downstream of hematopoietic stem cells (HSC) and multipotent progenitors (MPP) are common myeloid progenitors (CMPs) and common lymphoid progenitors (CLPs). CMPs generate myeloid or erythroid cells while CLPs give rise to lymphoid cells exclusively. This model suggests the thymus is settled by lymphoid-restricted CLPs possessing T, B, and NK lineage potentials, but devoid of non-lymphoid lineage potentials. The revised model (bottom) proposes the existence of lymphoid-myeloid progenitors (LMPs, originally described by Katsura and colleagues [2,3]) as a developmental intermediate in T, B, and myeloid development. Accordingly, this model proposes the thymus is settled by lymphoid-myeloid progenitors with developmental potentials for myeloid, T, and additional cell lineages.

Figure 2. Current view of T lymphocyte development

Progenitors with T cell lineage potential in bone marrow and thymus are depicted. Key non-T cell lineage potentials are indicated at top and bottom in gray. Many progenitor cell types in BM can respond to Notch1 signaling and so possess T lineage potential. Some of these progenitors share an LSK ($\text{Lin}^- \text{Sca-1}^+ \text{Kit}^+$) phenotype, but can be distinguished by surface expression of the Flt3 receptor. They include $\text{Flt3}^- \text{LSK}$ populations enriched in hematopoietic stem cells (HSC), $\text{Flt3}^{\text{low}} \text{LSK}$ multipotent progenitors (MPP), and $\text{Flt3}^{\text{hi}} \text{LSK}$ early lymphoid progenitors (ELP) that also transcribe lymphoid genes such as RAG. Certain progenitors outside the LSK compartment also possess T potential, including $\text{Lin}^- \text{Flt3}^+ \text{IL7R}^{\text{hi}} \text{Kit}^{\text{low}} \text{Sca-1}^{\text{low}}$ common lymphoid progenitors (CLP as well as downstream B220^+ CLP-2) and committed T cell progenitors (CTPs). Whereas many cells have T potential, not all progenitors can home to the thymus effectively. Instead, the ability to home to the thymus is selective, involving molecules such as PSGL-1 and CCR9. While PSGL-1 is not known to be differentially expressed among those BM progenitors described here, CCR9 is first expressed at the ELP stage. Erythroid potential is absent by the ELP stage, but myeloid potential is preserved in ELPs, and likely some CLPs as well as CLP-2 cells. Together, the collective group of ELPs, CLPs, and CLP-2 cells can be regarded functionally as LMPs mentioned in Figure 1, and depicted in green. This group of progenitors is suggested to home to and colonize the thymus as thymus-settling progenitors (TSPs). Consistently, ETPs possess myeloid and lymphoid lineage potentials. As ETPs differentiate into downstream progenitors, cells receive more Notch1 signals, as evidenced by increasing expression of Notch1 target genes. Concomitantly, the ability to differentiate into alternative lineages is diminished and ultimately lost by the DN3 stage. Note that TSPs may be very heterogeneous. It is possible that some TSPs can bypass the ETP stage. Detailed phenotypes for these progenitor populations have been recently published [36].