

## Thymus-bound: the many features of T cell progenitors

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## 1. ABSTRACT

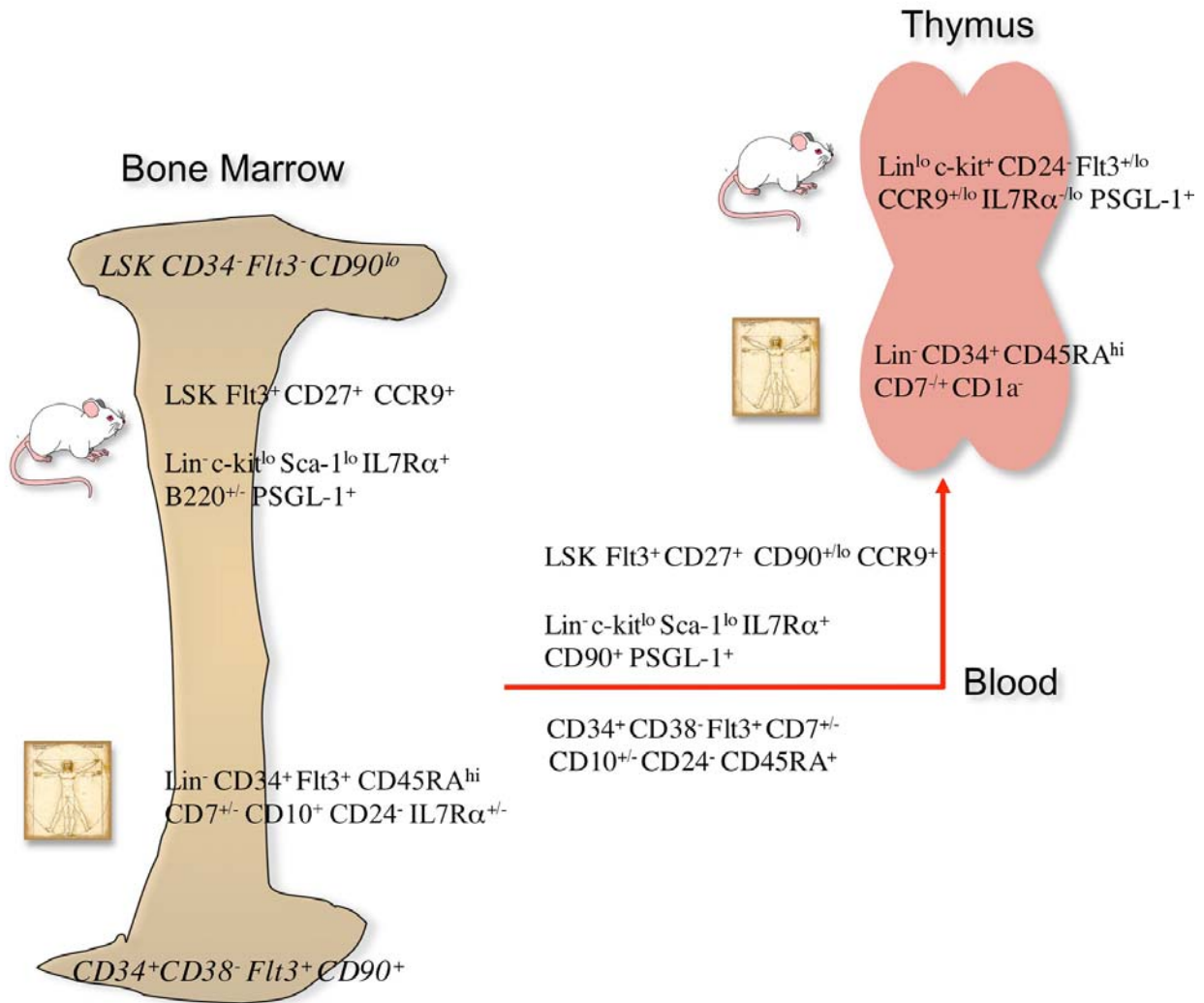
T cells are unique in that they begin their development as a progenitor within the bone marrow but complete their differentiation within the thymus. Furthermore, long-term T-lymphopoiesis requires a continuous supply of thymus-bound progenitors derived from the bone marrow. The critical role for T cells is clearly observed in individuals with genetic or acquired immunodeficiencies or those having undergone hematopoietic stem cell transplantation. Here, we review the work done by several groups aimed at characterizing the earliest T-lineage progenitors (ETPs), in mouse and human, found within the thymus, in addition to the long-sought after thymus-colonizing progenitor, which makes its journey from the bone marrow via the bloodstream into thymus. The characterization of these progenitors may herald therapeutic insight into the restoration of T cells in immunodeficient individuals.

## 2. INTRODUCTION

The hematopoietic system is composed of a plethora of cell types that differ in abundance, differentiation potential, proliferation and effector-function. Hematopoietic stem cells (HSCs) are rare, bone marrow (BM) resident primitive cells that are unique in that they have high self-renewal capacity plus the potential to differentiate into all of the blood-borne cell lineages. The downstream progeny of HSCs are more abundant and lack long-term or life-long self-renewal potential.

### 2.1. Mouse and Human Hematopoiesis

The markers used to define human and mouse HSCs, in addition to their downstream successors, differ vastly (Figure 1). Mouse HSCs are CD34<sup>+</sup> Flt3/CD135<sup>-</sup> Thy1.1/CD90<sup>lo</sup> and reside within the lineage-marker negative (Lin<sup>-</sup>) Sca-1<sup>+</sup> c-Kit/CD117<sup>+</sup> (LSK) fraction of BM cells (1-3). Downstream of the HSC, but within the LSK



**Figure 1.** Schematic representation of human and mouse intra- and extra-thymic T-lineage progenitors. A composite and generic phenotypic characterization for likely progenitor cells, present in the bone marrow, blood, and thymus, which have been shown to possess T-lineage differentiation potential and/or thymus-homing abilities are represented for mouse and human model systems.

population, lies the multipotent progenitor (MPP), which is short-term renewing and expresses Flt3, the Flt3-ligand receptor. Advances in the field of human HSC biology have been aided by the use of *in vitro* clonogenic/stromal assays and more recently by improved xenotransplantation mouse models. Using these assays, human HSCs have been found within the CD34<sup>+</sup> CD38<sup>-</sup> fraction of BM cells. CD34 is also present on downstream precursor cells specified to other lineages but not on mature differentiated hematopoietic cells. As HSCs differentiate they acquire CD38 on the cells surface and lose expression of CD34. Thus, long-term HSCs have been identified as CD34<sup>+</sup> CD38<sup>-</sup> Flt3<sup>+</sup> CD90<sup>lo</sup> (4-9) cells.

Hematopoiesis is thought to operate hierarchically, whereby pluripotent HSCs differentiate via successive restrictions of cell-fate potential into more lineage-restricted cells. The prospective isolation of cell subsets from the blood and BM based on cell surface

antigens has allowed for the generation of a developmental hierarchy starting from HSCs to downstream lineage-restricted progenitor cells. Key branch points in this hierarchy include whether an HSC-derived cell will differentiate along the lymphoid lineage to produce T, B and NK cells or along the erythromyeloid lineage to produce red cells, platelets, monocytes and granulocytes. The original model (10) posits that HSC differentiation occurs along one of the aforementioned two pathways via a strict separation into a Lin<sup>-</sup> Sca-1<sup>lo</sup> c-kit<sup>lo</sup> IL7Rα/CD127<sup>+</sup> common lymphoid precursor (CLP) and a common myeloid precursor (CMP) to generate T, B and NK cells or monocytes/macrophages and granulocytes, respectively. However, evidence from Jacobsen's group demonstrated the presence of an LSK CD34<sup>+</sup> Flt3<sup>+</sup> lymphoid-primed multipotent progenitor (LMPP) in mouse BM that possesses combined granulocyte, macrophage, T cell and B cell potential with attenuated megakaryocytic and erythroid

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potential (11). Thus, the issue of whether there is a strict separation of lymphoid and myeloid progenitors remains open, but recent evidence, discussed herein, points in support of this alternate model of hematopoietic lineage differentiation, which contains the progenitors that would eventually give rise to T cells within the thymus.

### 3. MOUSE EARLY T-LINEAGE PROGENITORS

Unlike all the other hematopoietic lineages, T cells are unique in that they undergo development within the thymus. T-lymphocytes undergo a series of coordinated developmental transitions, with the most immature cells within the mouse thymus being double negative (DN) for the expression of CD4 and CD8 (CD4<sup>-</sup>CD8<sup>-</sup>) (12). DN cells can be further discriminated into CD44<sup>+</sup>CD25<sup>-</sup>, CD44<sup>+</sup>CD25<sup>+</sup>, CD44<sup>-</sup>CD25<sup>+</sup>, and CD44<sup>-</sup>CD25<sup>-</sup> subsets, named DN1, DN2, DN3 and DN4, respectively. In addition, the DN1 population is heterogeneous and can be further fractionated into DN1a-DN1e subsets based on CD117 and CD24 expression (13). The most immature thymocytes are IL7R $\alpha$ <sup>-low</sup> and reside within the DN1a/DN1b subsets, also termed early T-lineage progenitors or ETPs (14, 15). Numerous studies have aimed at characterizing this population more extensively. Characterization by Sambandam *et al.* (16) demonstrated the presence of a more immature Flt3<sup>+</sup> ETP population and more mature Flt3<sup>lo</sup> population, representing ~10% and ~90% of ETPs, respectively. Further characterization of ETPs using a CCR9-EGFP knock-in reporter mouse demonstrated that ETPs express CCR9 (17), which led to further refinement of these cells as either Flt3<sup>+</sup>CCR9<sup>+</sup> or Flt3<sup>lo</sup>CCR9<sup>lo</sup> (18).

Given the critical role of Notch in T cell development (19), it was not surprising that ETPs are responsive to Notch signals in that they express Notch target gene transcript upregulation compared to BM LSK cells. In addition, Flt3<sup>+</sup> ETPs downregulate Flt3 upon Notch activation and upregulate CD25 to progress to the DN2 stage of T cell development. Also, when HSCs retrovirally-expressing dnMAML (an inhibitor of Notch signaling) were injected into irradiated mice, little to no ETP<sup>s</sup> were detected in the GFP<sup>+</sup> (dnMAML-expressing) subset of thymocytes, thus providing further evidence for the role of Notch in ETP generation (16).

Representing only 0.01% of thymocytes in an adult mouse and exhibiting robust T cell progenitor activity, ETPs (15) are capable of generating multiple non-T lineage fates such as NK, DC, and myeloid cells, however they exhibit weak B cell potential (20). Whether some of these other lineages arise from a single common T-progenitor or independently from a different type of T-progenitor subset or from non-T-progenitors remains controversial. In contrast to reports demonstrating the absence of B cell potential in ETPs (13, 20), using the CCR9 eGFP knock-in reporter mouse it was reported that ETPs possess both T and B cell potential at the clonal level when assayed on a mixture of OP9/OP9-DL1 cell monolayers (17, 21). An additional study reported weak B cell potential from Flt3<sup>+</sup> ETPs and not Flt3<sup>lo</sup> ETPs when injected intravenously into irradiated mice (16).

With regards to other lineages, e.g., thymic macrophages arising from an early T cell precursor, the original model of hematopoiesis would suggest these cells arise from an independently migrated myeloid progenitor from the circulation. When Wada *et al.*, assayed DN1 thymocytes at a clonal level, this population lacked B cell potential but retained strong macrophage, NK and DC cell fate potential (22). Furthermore, the appearance of bipotent T/macrophage progenitors was also observed. Specifically examining ETPs, Bell and Bhandoola were able to generate phagocytic CD11b<sup>+</sup> cells upon bulk culture of ETPs on OP9 cells (23). When the bipotential ability of ETPs was assessed at the clonal level it was revealed that a high frequency of cells possessed both T and myeloid potential. These studies demonstrate a shared progenitor for T-lineage and myeloid cells in the thymus, suggesting that a binary split in the T-myeloid cell fates prior to thymus entry is likely inaccurate. However, a contrasting report arguing for the original model of hematopoiesis was recently published by Schlenner *et al.*, (24), in which an IL7R $\alpha$  cre-recombinase knock-in mouse was used to trace the cell-lineage history of cells that expressed or previously expressed IL7R, and thus denoting a lymphoid origin. Examination of the thymus from these mice revealed the large majority but not all myeloid cells were derived from IL7R<sup>-</sup> cells, i.e., progenitors that never expressed IL7R unlike those that would typically give rise to T-lineage cells. Additionally, when IL7R<sup>-</sup> or IL7R<sup>+</sup> DN1 cells were injected intrathymically into irradiated recipients, T cells were robustly generated while donor-derived macrophages were not detected. Of note, both groups, when using VDJ reporter or IL7R reporter mice (23, 24), observed a significant proportion of thymic granulocytes that were likely derived from lymphoid progenitors.

The capacity of ETPs to generate alternative lineages is tightly controlled by the expression of transcription factors, with lineage outcomes ultimately determined by the balance of these regulatory factors (25). During early T-lineage differentiation, in addition to Notch signaling, GATA-3, Bcl11b, and E-proteins become up-regulated to specify ETPs toward the T cell fate, with TCF1 (Tcf7) playing a role during T cell commitment (26). The balance of transcriptional regulators and the plasticity and/or multipotential nature of immature T cell progenitors (27-29) were recently highlighted in studies using Bcl11b<sup>-/-</sup> mice. Immature T cells from Bcl11b<sup>-/-</sup> mice were found to arrest at the DN2 stage of development and were converted into induced NK cells, which possessed cytolytic function and could clear tumors *in vivo*. Thus withdrawal of this transcription factor was able to reveal the alternate lineage capacity of early thymocytes.

### 4. MOUSE EXTRATHYMIC T CELL PROGENITORS

As ETPs do not have intrinsic self-renewal capacity, T cells, like all other blood-lineage cells, are ultimately derived from an HSC in the bone marrow, which gives rise to a progenitor cell that enters the circulation bound for the thymus. The precise identity of this thymus-settling cell remains unknown, however numerous studies

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have been performed to determine the phenotype of this population. As such, both BM and blood have been closely examined for cells possessing robust T-lineage differentiation potential.

Multiple hematopoietic progenitors from BM have been identified that show T-lineage potential (18). Indeed, even BM-derived HSCs give rise to T cells when placed intrathymically (15, 30), however these cells are not found within the thymus, as these cells fail to home to the thymus (31), and thus are an unlikely source of physiological thymus-seeding progenitors. Downstream of the HSC, MPPs, LMPPs and CLPs appear to be more attractive candidates to contain a cell that is thymus bound. Using a *Rag1*-GFP knock-in mouse, to identify lymphoid-restricted populations, a subset of LSK Flt3<sup>+</sup> CD27<sup>+</sup> GFP<sup>+</sup> MPPs were detected and referred to as early lymphoid progenitors (ELPs) (32). ELPs display a significant loss of megakaryocyte and erythroid potential and show robust T cell potential (in addition to B and NK cell potential) when injected into *Rag1*<sup>-/-</sup> animals. Both CLP and its B220<sup>+</sup> progeny, CLP-2, possess thymus-seeding capacity *in vivo* (10, 33), with CLP-2 cells exhibiting higher T cell potential. In accordance with the differentiation status of the candidate progenitors, CLPs reconstitute mouse thymus with faster kinetics and in a single or limited wave of differentiation as compared to MPPs (10, 34). Using depletion, rather than an enrichment strategy, two groups recently verified that thymus repopulating activity is found within the Flt3<sup>+</sup> CD27<sup>+</sup> compartment of BM (34, 35), which includes CLPs and MPPs, and further supports the notion that different types of progenitor cells can function as thymus-seeding cells (18).

With blood being the thoroughfare between the BM and thymus, potential T-lineage progenitors must be capable of migration. This requirement dictates the need for receptors involved in homing and adhesion to be expressed on thymus-colonizing subsets. High expression levels of the chemokine CCL25, by the thymus stromal cells, make cells bearing its receptor, CCR9, an obvious candidate for having thymus-settling activity. Using a CCR9-eGFP knock in mouse to identify T-precursors *in vivo*, CCR9<sup>+</sup> cells were found in the DN1 fraction of thymocytes (17), and thus an incoming progenitor is likely to express this receptor. Indeed, all thymus-repopulating activity resided within the eGFP-CCR9<sup>+</sup> LSK fraction of BM, however some eGFP-CCR9<sup>+</sup> fractions lacked T cell potential, thus precluding some subpopulations. Moreover, eGFP-CCR9<sup>+</sup> LSKs that possessed T-lineage potential were detected at extremely low levels in the blood (17), which further highlights the rarity of this sought-after cell type. Early work demonstrated that CCR9<sup>-/-</sup> mice display normal T cell development but when tested in a competitive setting *in vivo*, CCR9<sup>-/-</sup> cells were inefficient at repopulating the thymus compared to wild-type competitors (31, 36, 37). An extension of this work revealed that CCR9 expression is absent on HSCs, while present on a subset of MPP and CLPs. In addition, mice reconstituted with CCR9<sup>-/-</sup> BM generated significantly less ETPs. Recent studies, published simultaneously, now demonstrate a cooperative role for both CCR7 and CCR9 in thymus-settling (38, 39).

Zlotoff *et al.*, found a rare population of CCR7<sup>+</sup> CCR9<sup>+</sup> co-expressing cells in the BM of adult mice confined to the LMPP and CLP subsets. CCR7<sup>-/-</sup> mice have normal T cell development, slightly lower thymic cellularity and display a slight reduction in ETP cellularity upon competitive transfer of CCR7<sup>-/-</sup> BM cells. In contrast, double-knockout mice (DKO) (CCR7<sup>-/-</sup> CCR9<sup>-/-</sup>) exhibited reduced thymic cellularity and displayed a near absence of ETPs and DN2 thymocytes. The near absence of ETPs with only a modest reduction in total thymus cellularity indicates a compensatory mechanism within the thymus for maintenance of thymic numbers. In addition, Krueger *et al.*, (39) sought to determine whether the phenotype observed in DKO mice was due to a defect in the generation or release of BM-derived precursors in BM or into blood, however normal frequencies of MPPs and CLPs was observed.

The search for blood-borne progenitors has proven more difficult due to the rarity of and likely short half-life of these cells within the circulation. Some groups have identified rare circulating thymic progenitors (CTPs) in fetal blood that possess a Lin<sup>-</sup> c-kit<sup>lo</sup> Thy1<sup>+</sup> phenotype that displayed T-lineage potential when placed intrathymically. Furthermore, CTPs were present in athymic nude mice, demonstrating a thymus-independent mode of generation (40, 41). More recently CTPs have been found in adult mice with the use of pTα-hCD25 reporter transgenic mice to identify lymphoid-restricted cells. CTPs that were Lin<sup>h</sup>CD25<sup>+</sup> B220<sup>-</sup> lacked B and myeloid cell potential, but displayed a single wave of T cell colonizing activity when injected in Rag2<sup>-/-</sup>γc<sup>-</sup> mice. In addition, CTPs expressed CCR9 and another molecule thought to play an important role in thymic recruitment (42), platelet-selectin (P-selectin) glycoprotein ligand-1 (PSGL-1). PSGL1 is expressed by BM lymphoid progenitors and its receptor is expressed on thymic endothelium. Similar to CCR9<sup>-/-</sup> mice, PSGL1 deficient mice do not have any obvious defects in T cell development. However, the ETP compartment was reduced compared to wild-type (WT) mice (42). This finding suggested there were unoccupied thymic niches more readily available to accept incoming progenitors than an occupied one. Indeed, WT BM injected into PSGL1<sup>-/-</sup> mice displayed higher levels of donor-derived cells in the thymus than those injected into WT mice. Of high significance was the feedback loop that was demonstrated between thymic occupancy and P-selectin levels expressed on thymic stroma. The authors demonstrate that mice with reduced thymic occupancy (IL7R<sup>null</sup> and PSGL1<sup>-/-</sup> mice) expressed high P-selectin levels, which was in stark contrast to WT mice. Thus, a clear link was provided between progenitor recruitment, the availability of thymic niches and P-selectin as the gate-keeper for immigrant progenitors. Indeed, multiple lines of evidence suggest that the export of progenitor cells from the bone marrow is coordinated with the ability of the thymus to accept new immigrants and does so in a periodic fashion (42-44).

While the majority of studies examining progenitor T cells are performed on adult tissues, the analysis of fetal specimens for the identification of the

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earliest T cell progenitor also proved insightful. The colonization of mouse fetal thymus by fetal liver progenitors occurs at embryonic day (E) 11.5 prior to thymus vascularization (45). During the pre-vascularization period, T cell progenitors enter the thymus from the blood through a layer of perithymic mesenchyme. Indeed, similar to adults, the CCR7/CCL21 and CCR9/CCL25 chemokine axis was shown to play a major role in fetal thymocyte recruitment prior to vascularization. Liu *et al.* demonstrate that mice deficient for either CCR7 or CCR9 had a modest reduction in fetal thymocytes, however double-deficient mice exhibited a more severe reduction in E14.5 thymus cellularity. Furthermore, a similar reduction of cells was found within the perithymic region, but not within fetal liver or circulation, suggesting a defect in recruitment from the circulation to the thymus (46). Since some fetal thymocytes are still observed in double-deficient animals, the expression of the chemokine receptor CXCR4, involved in adult intrathymic migration, was examined (47). While CXCR4 was clearly present on the majority of hematopoietic progenitors within fetal liver, it was present on less than half of the cells within the perithymic mesenchyme, and was absent on progenitor cells found within the thymic epithelium. Thus a clear role of CXCR4 has not yet been established for fetal thymocyte recruitment.

### 5. HUMAN EARLY T-LINEAGE PROGENITORS

While mouse hematopoiesis and the identity of T-lineage progenitors are becoming more refined, candidate populations in humans still warrant further characterization. Nevertheless, some insights into the phenotype of human ETPs and extrathymic precursors have been recently attained. Like mouse thymocytes, human T-lymphopoiesis begins with precursors that are triple negative for CD3, CD4 and CD8. Pioneering work from Barton Haynes' group demonstrated that CD34<sup>+</sup> CD7<sup>+</sup> cells are present in human thymus, and thus represent early thymocyte progenitors (48, 49). The findings that CD7 is one of the earliest markers to appear in human T cell ontogeny and that immature thymocytes are contained within the CD34<sup>+</sup> CD7<sup>+</sup> CD1a<sup>-</sup> population have been confirmed by numerous groups (50-53). A detailed analysis by Hao *et al.*, however, revealed that a rare population (0.2%) of Lin<sup>-</sup> CD34<sup>+</sup> CD7<sup>-</sup> cells also exists within human thymus (54). Furthermore, several groups have established that the most primitive cells in the thymus are multipotential in their ability to proceed along T, NK cell, plasmacytoid dendritic cell and myeloid differentiation (53, 55-59). However, unlike mouse thymocytes the most immature T-progenitors in the human thymus also exhibit erythroid potential, with clonal assays demonstrating B, NK, myeloid and erythroid potential of this population (54, 60). The ability to give rise to erythroid cells highlights the more primitive nature of human ETPs - compared to mouse ETPs -, alluding to either the possible presence of an HSC/MPP within this subset or a true developmental intermediate between the two subsets. Although HSCs can differentiate into T cells when placed directly into the thymus (61), it is an unlikely candidate for the physiological human thymus-seeding progenitor (31), as

the major chemokine axis (CCL25 & CCL21) guiding progenitors toward the thymus likely has little effect on circulating HSC levels due to low or absent CCR9 and CCR7 cell surface expression.

### 6. HUMAN EXTRATHYMIC T CELL PROGENITORS

Elucidation of the ETP phenotype and lineage potential have helped to refine the search within human adult and fetal BM, and umbilical cord blood (UCB) for putative thymus seeding cells. During human fetal life, progenitor cells colonizing the thymus via the blood are derived first from fetal liver (FL) around gestational week 8-9 followed by bone marrow from week 22 onwards through adulthood. In one study, fetuses were examined at week 7 prior to thymic colonization, and it was found that the yolk sac, upper neck, and thorax contained CD7<sup>+</sup> cells. Of note, while some of these sites were devoid of CD7<sup>+</sup> cells by gestational week 9.5, these cells were now present in the newly formed thymus (49). An extensive study of human fetal tissues by Haddad *et al.*, (52) detected a CD34<sup>hi</sup> CD45RA<sup>hi</sup> CD7<sup>+</sup> population in bone marrow as early as the onset of colonization by FL HSCs. This population was never found in FL, suggesting, that the BM is the primary site for the production of progenitors that colonize the human thymus during gestation. These authors observed that bone marrow derived CD34<sup>hi</sup> CD45RA<sup>hi</sup> CD7<sup>+</sup> cells gave rise to T cells in fetal thymus organ cultures (FTOC) and confirmed the ability of these cells to enter thymus parenchyma in an *ex vivo* colonization assay.

While human CLP and LMPP have remained enigmatic, some studies have shed insight into the phenotype of these populations. Early work aimed at characterizing putative T cell progenitors in the BM used terminal deoxynucleotidyl transferase (TdT) as a marker for lymphoid progenitors (50, 62, 63). Indeed, CD34<sup>+</sup> TdT<sup>+</sup> cells were identified in the BM that lacked the majority of mature surface markers, however ~50% were also CD10<sup>+</sup>. Galy *et al.*, were the first to demonstrate candidate CLPs in human BM (64). Lin<sup>-</sup> CD34<sup>+</sup> CD10<sup>+</sup> CD45RA<sup>+</sup> were detected in adult BM and possessed the ability to give rise to B, NK, and DC cells at the single level cell. T cell differentiation was observed upon implantation of Lin<sup>-</sup> CD34<sup>+</sup> CD10<sup>+</sup> CD45RA<sup>+</sup> microinjected FTOCs implanted into immunodeficient mice. In keeping with the definition of CLPs, this population lacked myeloid, megakaryocytic and erythroid potential. A candidate CLP population identified as CD34<sup>+</sup> CD38<sup>-</sup> CD7<sup>+</sup> CD45RA<sup>+</sup> was also found in UCB. While its CD10<sup>+</sup> CD7<sup>-</sup> counterpart possessed clear myeloid potential in methylcellulose assays, the CD7<sup>+</sup> population demonstrated only B, NK and DC potential, thus representing putative myeloid- and lymphoid-restricted progenitors. Similar findings were observed by Hoebeke *et al.*, and extended the aforementioned work by confirming T cell differentiation of the CD34<sup>+</sup> CD38<sup>-</sup> CD7<sup>+</sup> subset from UCB (65). They also performed an extensive gene expression analysis and demonstrated the upregulation of multiple genes involved in T and B lymphoid development and downregulation of myeloid-associated

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genes. In contrast to mouse CLPs, this human candidate population was devoid of IL7R $\alpha$  cell surface expression. It should be noted that Ryan *et al.*, was able to detect IL7R $\alpha$  expression on CD34<sup>+</sup> Lin<sup>-</sup> cells isolated from human BM that exhibited B cell potential but lacked myeloid potential (66). Another candidate human CLP described by Six *et al.*, further characterized the Lin<sup>-</sup> CD34<sup>+</sup> CD10<sup>+</sup> fraction of UCB and BM and found that this subset could be broken down based on CD24 expression (67). They observed that the CD10<sup>+</sup> CD24<sup>-</sup> subset possessed B, T, and NK cell potential but lacked strong myelo-erythroid activity. In contrast, the CD10<sup>+</sup> CD24<sup>+</sup> population appeared to be B-lineage restricted, as it was unable to give rise to any other lineages and expressed B cell specific genes. Unlike the aforementioned studies, which failed to detect myeloid potential in multiple candidate populations in CB and BM, Doulatov *et al.*, recently described in UCB a mode of lympho-myeloid segregation in human CB and BM that does not follow the original murine model (68). The authors extensively characterized seven populations for lymphoid, myeloid, granulocyte, and megakaryocyte/erythroid (Meg/E) potential from UCB, based on CD34, CD38, Thy-1, CD45RA, Flt3, CD10 and CD7 expression. These populations were rigorously tested for lineage potential in bulk and clonally, on MS-5 stromal cells, colony forming unit (CFU) assays, OP9-DL1 cells, and in immunodeficient mice. Of particular interest were two fractions - CD34<sup>+</sup> CD38<sup>-</sup> CD90<sup>neg/lo</sup> CD45RA<sup>+</sup> Flt3<sup>+</sup> CD10<sup>+</sup> CD7<sup>-</sup> and CD34<sup>+</sup> CD38<sup>-</sup> CD90<sup>neg/lo</sup> CD45RA<sup>+</sup> Flt3<sup>+</sup> CD10<sup>+</sup> CD7<sup>+</sup> cells that exhibited multi-lymphoid (MLP) potential with regards to T, B, NK and DC generation. In addition to lymphoid potential, both fractions possessed strong myeloid potential. Although Haddad *et al.*, demonstrated a B-cell bias in CD34<sup>+</sup> CD45RA<sup>+</sup> CD10<sup>+</sup> progenitors from UCB compared to CD7<sup>+</sup> progenitors (69), the MLP fractions described by Doulatov *et al.* showed similar B cell and lympho-myeloid potential regardless of CD7 expression on the CD10<sup>+</sup> fractions. Additionally, both fractions displayed a clear loss of Meg/E potential in CFU assays and *in vivo*. Furthermore, when as few as 1000 MLPs were injected into NOD/SCID/ $\gamma$ c<sup>null</sup> mice, CD19<sup>+</sup> B cells and CD33<sup>+</sup> myeloid cells are clearly observed. Unfortunately, the ability of these cells to colonize the thymus was not assessed due to the early time-points analyzed for B and myeloid potential. Nevertheless, these subsets showed clear T-lineage potential *in vitro*, using the OP9-DL1 cell assay (70). Of note, these two MLP populations were devoid of granulocyte potential. Instead granulocyte potential was found downstream of a CMP subset. Thus, while these authors do not observe a rigid separation of lymphoid and myeloid cell fates, they find a CMP that follows the classical model of restriction into a granulocyte/macrophage progenitor and Meg/E progenitor similar to that demonstrated in mouse.

## 7. CONCLUSION

Over the years, numerous studies have helped to elucidate the phenotype, functional nature, and lineage potential of early T-lineage progenitors. Although, the nature of these cells in the mouse is not fully elucidated, it is becoming more refined. With respect to human T-

lymphopoiesis, this level of definition had been lacking, due to the model system and ethical considerations. However, the availability of immunodeficient mouse strains that engraft human HSCs and progenitor cells, as well as stromal cells, such as OP9-DL1, have permitted a closer inspection of T-lymphocyte development at the bulk, and, more importantly, at the clonal level. Although several important strides are being made with respect to human lymphopoiesis and thymus-seeding progenitors, mouse strains still need to be further humanized (human cytokines, human MHC) in order to reveal outcomes that may not be determined from current strains.

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**Abbreviations:** BM, bone marrow; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; CTP, circulating thymic progenitor; CFU, colony forming units; DKO, double knock-out; DN, double negative; E, embryonic day; ELP, early lymphoid progenitor; ETP, earliest T cell progenitor; FL, fetal liver; FTOC, fetal thymus organ culture; HSC, hematopoietic stem cell; Lin, lineage; LMPP, lymphoid-primed multipotent progenitor; LSK, Lineage<sup>-</sup> Sca1<sup>+</sup> cKit<sup>+</sup>; Meg/E, megakaryocytic/erythroid; MLP, multi-lymphoid progenitor; MPP, multipotent progenitor; PSGL-1, P-selectin glycoprotein ligand-1; Tdt, terminal deoxynucleotidyl transferase; UCB, umbilical cord blood; WT, wild-type.

**Key Words:** Thymus, T-lymphopoiesis, ETP, MPP, CLP, Progenitors, Hematopoiesis, Notch, Review

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