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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 $CD34^-$ $Flt3/CD135^-$ $Thy1.1/CD90^{lo}$ and reside within the lineage-marker negative (Lin^-) $Sca\text{-}1^+$ $c\text{-}Kit/CD117^+$ (LSK) fraction of BM cells (1-3). Downstream of the HSC, but within the LSK

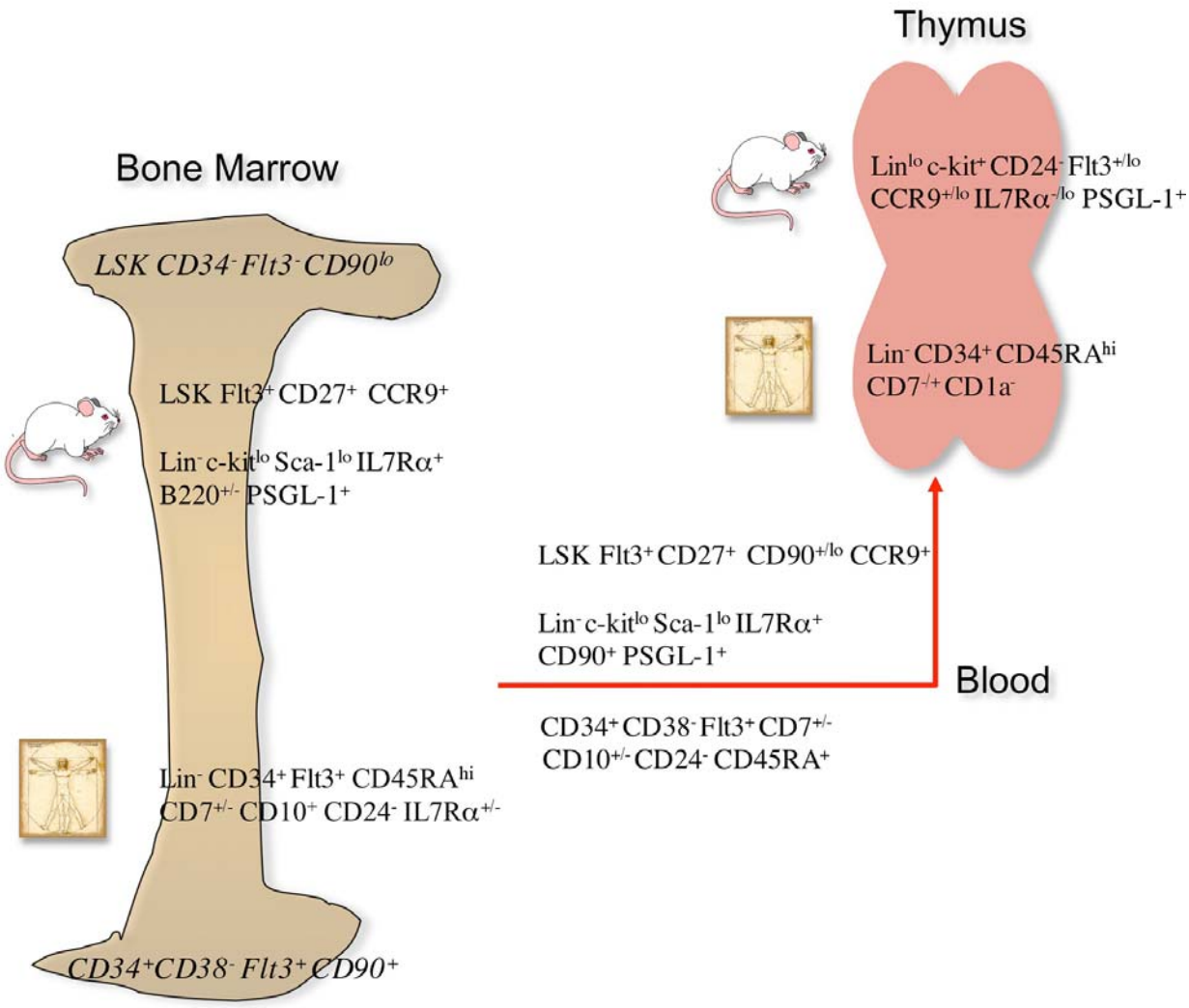

Figure 1. Schematic representation of human and mouse intra- and extra-thymic T-lineage progenitors. A composite and generic phenotypic characterization for likely progenitor cells, present in the bone marrow, blood, and thymus, which have been shown to possess T-lineage differentiation potential and/or 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 $CD34^+$ $CD38^-$ 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 $CD34^+$ $CD38^-$ $Flt3^+$ $CD90^{lo}$ (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^- $Sca\text{-}1^{lo}$ $c\text{-}kit^{lo}$ $IL7R\alpha/CD127^+$ 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 $CD34^+$ $Flt3^+$ lymphoid-primed multipotent progenitor (LMPP) in mouse BM that possesses combined granulocyte, macrophage, T cell and B cell potential with attenuated megakaryocytic and erythroid

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 ($CD4^-$ $CD8^-$) (12). DN cells can be further discriminated into $CD44^+$ $CD25^-$, $CD44^+$ $CD25^+$, $CD44^-$ $CD25^+$, and $CD44^-$ $CD25^-$ 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 thymoctyes are IL7R$\alpha^{-/low}$ 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 $Flt3^+$ ETP population and more mature $Flt3^{lo}$ 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 $Flt3^+$ $CCR9^+$ or $Flt3^{lo}$ $CCR9^{lo}$ (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, $Flt3^+$ 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's were detected in the GFP^+ (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 $Flt3^+$ ETPs and not $Fl3^{lo}$ 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 $CD11b^+$ 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 IL7Rα 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 $IL7R^-$ cells, i.e., progenitors that never expressed IL7R unlike those that would typically give rise to T-lineage cells. Additionally, when $IL7R^-$ or $IL7R^+$ 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 $Bcl11b^{-/-}$ mice. Immature T cells from $Bcl11b^{-/-}$ 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

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 $Flt3^+$ $CD27^+$ GFP^+ 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 $Rag1^{-/-}$ animals. Both CLP and its $B220^+$ 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 $Flt3^+$ $CD27^+$ 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*, $CCR9^+$ 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-$CCR9^+$ LSK fraction of BM, however some eGFP-$CCR9^+$ fractions lacked T cell potential, thus precluding some subpopulations. Moreover, eGFP-$CCR9^+$ 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 $CCR9^{-/-}$ mice display normal T cell development but when tested in a competitive setting *in vivo*, $CCR9^{-/-}$ 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 $CCR9^{-/-}$ 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 $CCR7^+$ $CCR9^+$ co-expressing cells in the BM of adult mice confined to the LMPP and CLP subsets. $CCR7^{-/-}$ mice have normal T cell development, slightly lower thymic cellularity and display a slight reduction in ETP cellularity upon competitive transfer of $CCR7^{-/-}$ BM cells. In contrast, double-knockout mice (DKO) ($CCR7^{-/-}$ $CCR9^{-/-}$) 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 at 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^- $c\text{-}kit^{lo}$ $Thy1^+$ 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^-hCD25^+ $B220^-$ lacked B and myeloid cell potential, but displayed a single wave of T cell colonizing activity when injected in $Rag2^{-/-}\gamma c^-$ 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 $CCR9^{-/-}$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 $PSGL1^{-/-}$ 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 ($IL7R^{null}$ and $PSGL1^{-/-}$ 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

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 $CD34^+$ $CD7^+$ 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 $CD34^+$ $CD7^+$ $CD1a^-$ 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^- $CD34^+$ $CD7^-$ 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 $CD7^+$ cells. Of note, while some of these sites were devoid of $CD7^+$ 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 $CD34^{hi}$ $CD45RA^{hi}$ $CD7^+$ 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 $CD34^{hi}$ $CD45RA^{hi}$ $CD7^+$ 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, $CD34^+$ TdT^+ cells were identified in the BM that lacked the majority of mature surface markers, however ~50% were also $CD10^+$. Galy *et al.,* were the first to demonstrate candidate CLPs in human BM (64). Lin^- $CD34^+$ $CD10^+$ $CD45RA^+$ 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^- $CD34^+$ $CD10^+$ $CD45RA^+$ 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 $CD34^+$ $CD38^-$ $CD7^+$ $CD45RA^+$ was also found in UCB. While its $CD10^+$ $CD7^-$ counterpart possessed clear myeloid potential in methylcellulose assays, the $CD7^+$ 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 $CD34^+$ $CD38^-$ $CD7^+$ 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

genes. In contrast to mouse CLPs, this human candidate population was devoid of IL7Rα cell surface expression. It should be noted that Ryan *et al.*, was able to detect IL7Rα expression on $CD34^+$ Lin^- 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^- $CD34^+$ $CD10^+$ fraction of UCB and BM and found that this subset could be broken down based on CD24 expression (67). They observed that the $CD10^+$ $CD24^-$ subset possessed B, T, and NK cell potential but lacked strong myelo-erythroid activity. In contrast, the $CD10^+$ $CD24^+$ 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 - $CD34^+$ $CD38^-$ $CD90^{neg/lo}$ $CD45RA^+$ $Flt3^+$ $CD10^+$ $CD7^-$ and $CD34^+$ $CD38^-$ $CD90^{neg/lo}$ $CD45RA^+$ $Flt3^+$ $CD10^+$ $CD7^+$ 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 $CD34^+$ $CD45RA^+$ $CD10^+$ progenitors from UCB compared to $CD7^+$ progenitors (69), the MLP fractions described by Doulatov *et al.* showed similar B cell and lympho-myeloid potential regardless of CD7 expression on the $CD10^+$ 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/γc^{null} mice, $CD19^+$ B cells and $CD33^+$ 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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9. REFERENCES

1. G. J. Spangrude, S. Heimfeld and I. L. Weissman: Purification and characterization of mouse hematopoietic stem cells. *Science* 241, 58-62 (1988)

2. S. J. Morrison and I. L. Weissman: The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. *Immunity* 1, 661-73 (1994)

3. M. Osawa, K. Hanada, H. Hamada and H. Nakauchi: Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* 273, 242-5 (1996)

4. C. M. Baum, I. L. Weissman, A. S. Tsukamoto, A. M. Buckle and B. Peault: Isolation of a candidate human hematopoietic stem-cell population. *Proc Natl Acad Sci U S A* 89, 2804-8 (1992)

5. M. Bhatia, J. C. Wang, U. Kapp, D. Bonnet and J. E. Dick: Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. *Proc Natl Acad Sci U S A* 94, 5320-5 (1997)

6. M. Bhatia, D. Bonnet, B. Murdoch, O. I. Gan and J. E. Dick: A newly discovered class of human hematopoietic cells with SCID-repopulating activity. *Nat Med* 4, 1038-45 (1998)

7. E. Sitnicka, N. Buza-Vidas, S. Larsson, J. M. Nygren, K. Liuba and S. E. Jacobsen: Human CD34+ hematopoietic stem cells capable of multilineage engrafting NOD/SCID mice express flt3: distinct flt3 and c-kit expression and response patterns on mouse and candidate human hematopoietic stem cells. *Blood* 102, 881-6 (2003)

8. H. Iwasaki and K. Akashi: Hematopoietic developmental pathways: on cellular basis. *Oncogene* 26, 6687-96 (2007)

9. K. J. Payne and G. M. Crooks: Human hematopoietic lineage commitment. *Immunol Rev* 187, 48-64 (2002)

10. M. Kondo, I. L. Weissman and K. Akashi: Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell* 91, 661-72 (1997)

11. J. Adolfsson, R. Mansson, N. Buza-Vidas, A. Hultquist, K. Liuba, C. T. Jensen, D. Bryder, L. Yang, O. J. Borge, L. A. Thoren, K. Anderson, E. Sitnicka, Y. Sasaki, M. Sigvardsson and S. E. Jacobsen: Identification of Flt3+ lympho-myeloid stem cells lacking erythro-megakaryocytic potential a revised road map for adult blood lineage commitment. *Cell* 121, 295-306 (2005)

12. R. Ceredig and T. Rolink: A positive look at double-negative thymocytes. *Nat Rev Immunol* 2, 888-97 (2002)

13. H. E. Porritt, L. L. Rumfelt, S. Tabrizifard, T. M. Schmitt, J. C. Zuniga-Pflucker and H. T. Petrie: Heterogeneity among DN1 prothymocytes reveals multiple progenitors with different capacities to generate T cell and non-T cell lineages. *Immunity* 20, 735-45 (2004)

14. K. Shortman and L. Wu: Early T lymphocyte progenitors. *Annu Rev Immunol* 14, 29-47 (1996)

15. D. Allman, A. Sambandam, S. Kim, J. P. Miller, A. Pagan, D. Well, A. Meraz and A. Bhandoola: Thymopoiesis independent of common lymphoid progenitors. *Nat Immunol* 4, 168-74 (2003)

16. A. Sambandam, I. Maillard, V. P. Zediak, L. Xu, R. M. Gerstein, J. C. Aster, W. S. Pear and A. Bhandoola: Notch signaling controls the generation and differentiation of early T lineage progenitors. *Nat Immunol* 6, 663-70 (2005)

17. C. Benz and C. C. Bleul: A multipotent precursor in the thymus maps to the branching point of the T versus B lineage decision. *J Exp Med* 202, 21-31 (2005)

18. A. Bhandoola, H. von Boehmer, H. T. Petrie and J. C. Zuniga-Pflucker: Commitment and developmental potential of extrathymic and intrathymic T cell precursors: plenty to choose from. *Immunity* 26, 678-89 (2007)

19. M. Ciofani and J. C. Zuniga-Pflucker: The thymus as an inductive site for T lymphopoiesis. *Annu Rev Cell Dev Biol* 23, 463-93 (2007)

20. G. Balciunaite, R. Ceredig and A. G. Rolink: The earliest subpopulation of mouse thymocytes contains potent T, significant macrophage, and natural killer cell but no B-lymphocyte potential. *Blood* 105, 1930-6 (2005)

21. K. Heinzel, C. Benz, V. C. Martins, I. D. Haidl and C. C. Bleul: Bone marrow-derived hemopoietic precursors commit to the T cell lineage only after arrival in the thymic microenvironment. *J Immunol* 178, 858-68 (2007)

22. H. Wada, K. Masuda, R. Satoh, K. Kakugawa, T. Ikawa, Y. Katsura and H. Kawamoto: Adult T-cell progenitors retain myeloid potential. *Nature* 452, 768-72 (2008)

23. J. J. Bell and A. Bhandoola: The earliest thymic progenitors for T cells possess myeloid lineage potential. *Nature* 452, 764-7 (2008)

24. S. M. Schlenner, V. Madan, K. Busch, A. Tietz, C. Laufle, C. Costa, C. Blum, H. J. Fehling and H. R. Rodewald: Fate mapping reveals separate origins of T cells and myeloid lineages in the thymus. *Immunity* 32, 426-36 (2010)

25. E. V. Rothenberg and D. D. Scripture-Adams: Competition and collaboration: GATA-3, PU.1, and Notch signaling in early T-cell fate determination. *Semin Immunol* 20, 236-46 (2008)

26. E. V. Rothenberg, J. E. Moore and M. A. Yui: Launching the T-cell-lineage developmental programme. *Nat Rev Immunol* 8, 9-21 (2008)

27. L. Li, M. Leid and E. V. Rothenberg: An early T cell lineage commitment checkpoint dependent on the transcription factor Bcl11b. *Science* 329, 89-93 (2010)

28. P. Li, S. Burke, J. Wang, X. Chen, M. Ortiz, S. C. Lee, D. Lu, L. Campos, D. Goulding, B. L. Ng, G. Dougan, B. Huntly, B. Gottgens, N. A. Jenkins, N. G. Copeland, F. Colucci and P. Liu: Reprogramming of T cells to natural killer-like cells upon Bcl11b deletion. *Science* 329, 85-9 (2010)

29. T. Ikawa, S. Hirose, K. Masuda, K. Kakugawa, R. Satoh, A. Shibano-Satoh, R. Kominami, Y. Katsura and H. Kawamoto: An essential developmental checkpoint for production of the T cell lineage. *Science* 329, 93-6 (2010)

30. R. Vicente, O. Adjali, C. Jacquet, V. S. Zimmermann and N. Taylor: Intrathymic transplantation of bone marrow-derived progenitors provides long-term thymopoiesis. *Blood* 115, 1913-20 (2010)

31. B. A. Schwarz, A. Sambandam, I. Maillard, B. C. Harman, P. E. Love and A. Bhandoola: Selective thymus settling regulated by cytokine and chemokine receptors. *J Immunol* 178, 2008-17 (2007)

32. H. Igarashi, S. C. Gregory, T. Yokota, N. Sakaguchi and P. W. Kincade: Transcription from the RAG1 locus marks the earliest lymphocyte progenitors in bone marrow. *Immunity* 17, 117-30 (2002)

33. C. H. Martin, I. Aifantis, M. L. Scimone, U. H. von Andrian, B. Reizis, H. von Boehmer and F. Gounari: Efficient thymic immigration of B220+ lymphoid-restricted bone marrow cells with T precursor potential. *Nat Immunol* 4, 866-73 (2003)

34. T. Serwold, L. I. Ehrlich and I. L. Weissman: Reductive isolation from bone marrow and blood implicates common lymphoid progenitors as the major source of thymopoiesis. *Blood* 113, 807-15 (2009)

35. N. Saran, M. Lyszkiewicz, J. Pommerencke, K. Witzlau, R. Vakilzadeh, M. Ballmaier, H. von Boehmer and A. Krueger: Multiple extrathymic precursors contribute to T-cell development with different kinetics. *Blood* 115, 1137-44 (2010)

36. S. Uehara, A. Grinberg, J. M. Farber and P. E. Love: A role for CCR9 in T lymphocyte development and migration. *J Immunol* 168, 2811-9 (2002)

37. M. Svensson, J. Marsal, H. Uronen-Hansson, M. Cheng, W. Jenkinson, C. Cilio, S. E. Jacobsen, E. Sitnicka, G. Anderson and W. W. Agace: Involvement of CCR9 at multiple stages of adult T lymphopoiesis. *J Leukoc Biol* 83, 156-64 (2008)

38. D. A. Zlotoff, A. Sambandam, T. D. Logan, J. J. Bell, B. A. Schwarz and A. Bhandoola: CCR7 and CCR9 together recruit hematopoietic progenitors to the adult thymus. *Blood* 115, 1897-905 (2010)

39. A. Krueger, S. Willenzon, M. Lyszkiewicz, E. Kremmer and R. Forster: CC chemokine receptor 7 and 9 double-deficient hematopoietic progenitors are severely impaired in seeding the adult thymus. *Blood* 115, 1906-12 (2010)

40. H. R. Rodewald, K. Kretzschmar, S. Takeda, C. Hohl and M. Dessing: Identification of pro-thymocytes in murine fetal blood: T lineage commitment can precede thymus colonization. *EMBO J* 13, 4229-40 (1994)

41. J. R. Carlyle and J. C. Zuniga-Pflucker: Requirement for the thymus in alphabeta T lymphocyte lineage commitment. *Immunity* 9, 187-97 (1998)

42. F. M. Rossi, S. Y. Corbel, J. S. Merzaban, D. A. Carlow, K. Gossens, J. Duenas, L. So, L. Yi and H. J. Ziltener: Recruitment of adult thymic progenitors is regulated by P-selectin and its ligand PSGL-1. *Nat Immunol* 6, 626-34 (2005)

43. K. Gossens, S. Naus, S. Y. Corbel, S. Lin, F. M. Rossi, J. Kast and H. J. Ziltener: Thymic progenitor homing and lymphocyte homeostasis are linked via S1P-controlled expression of thymic P-selectin/CCL25. *J Exp Med* 206, 761-78 (2009)

44. S. E. Prockop and H. T. Petrie: Regulation of thymus size by competition for stromal niches among early T cell progenitors. *J Immunol* 173, 1604-11 (2004)

45. C. C. Blackburn and N. R. Manley: Developing a new paradigm for thymus organogenesis. *Nat Rev Immunol* 4, 278-89 (2004)

46. C. Liu, F. Saito, Z. Liu, Y. Lei, S. Uehara, P. Love, M. Lipp, S. Kondo, N. Manley and Y. Takahama: Coordination between CCR7- and CCR9-mediated chemokine signals in prevascular fetal thymus colonization. *Blood* 108, 2531-9 (2006)

47. W. E. Jenkinson, S. W. Rossi, S. M. Parnell, W. W. Agace, Y. Takahama, E. J. Jenkinson and G. Anderson: Chemokine receptor expression defines heterogeneity in the earliest thymic migrants. *Eur J Immunol* 37, 2090-6 (2007)

48. J. Kurtzberg, S. M. Denning, L. M. Nycum, K. H. Singer and B. F. Haynes: Immature human thymocytes can be driven to differentiate into nonlymphoid lineages by cytokines from thymic epithelial cells. *Proc Natl Acad Sci U S A* 86, 7575-9 (1989)

49. B. F. Haynes, M. E. Martin, H. H. Kay and J. Kurtzberg: Early events in human T cell ontogeny. Phenotypic characterization and immunohistologic localization of T cell precursors in early human fetal tissues. *J Exp Med* 168, 1061-80 (1988)

50. L. W. Terstappen, S. Huang and L. J. Picker: Flow cytometric assessment of human T-cell differentiation in thymus and bone marrow. *Blood* 79, 666-77 (1992)

51. A. Galy, S. Verma, A. Bárcena and H. Spits: Precursors of CD3+CD4+CD8+ cells in the human thymus are defined by expression of CD34. Delineation of early events in human thymic development. *J Exp Med* 178, 391-401 (1993)

52. R. Haddad, F. Guimiot, E. Six, F. Jourquin, N. Setterblad, E. Kahn, M. Yagello, C. Schiffer, I. Andre-Schmutz, M. Cavazzana-Calvo, J. C. Gluckman, A. L. Delezoide, F. Pflumio and B. Canque: Dynamics of thymus-colonizing cells during human development. *Immunity* 24, 217-30 (2006)

53. H. Spits, L. L. Lanier and J. H. Phillips: Development of human T and natural killer cells. *Blood* 85, 2654-70 (1995)

54. Q. L. Hao, A. A. George, J. Zhu, L. Barsky, E. Zielinska, X. Wang, M. Price, S. Ge and G. M. Crooks: Human intrathymic lineage commitment is marked by differential CD7 expression: identification of CD7- lympho-myeloid thymic progenitors. *Blood* 111, 1318-26 (2008)

55. P. Res, E. Martínez-Cáceres, A. Cristina Jaleco, F. Staal, E. Noteboom, K. Weijer and H. Spits: CD34+CD38dim cells in the human thymus can differentiate into T, natural killer, and dendritic cells but are distinct from pluripotent stem cells. *Blood* 87, 5196-206 (1996)

56. B. Blom, P. Res, E. Noteboom, K. Weijer and H. Spits: Prethymic CD34+ progenitors capable of developing into T cells are not committed to the T cell lineage. *J Immunol* 158, 3571-7 (1997)

57. V. G. de Yebenes, Y. R. Carrasco, A. R. Ramiro and M. L. Toribio: Identification of a myeloid intrathymic pathway of dendritic cell development marked by expression of the granulocyte macrophage-colony-stimulating factor receptor. *Blood* 99, 2948-56 (2002)

58. C. Marquez, C. Trigueros, J. M. Franco, A. R. Ramiro, Y. R. Carrasco, M. Lopez-Botet and M. L. Toribio: Identification of a common developmental pathway for thymic natural killer cells and dendritic cells. *Blood* 91, 2760-71 (1998)

59. H. Spits, B. Blom, A. C. Jaleco, K. Weijer, M. C. Verschuren, J. J. van Dongen, M. H. Heemskerk and P. C. Res: Early stages in the development of human T, natural killer and thymic dendritic cells. *Immunol Rev* 165, 75-86 (1998)

60. F. Weerkamp, M. R. Baert, M. H. Brugman, W. A. Dik, E. F. de Haas, T. P. Visser, C. J. de Groot, G. Wagemaker, J. J. van Dongen and F. J. Staal: Human thymus contains multipotent progenitors with T/B lymphoid, myeloid, and erythroid lineage potential. *Blood* 107, 3131-7 (2006)

61. G. J. Spangrude and R. Scollay: Differentiation of hematopoietic stem cells in irradiated mouse thymic lobes. Kinetics and phenotype of progeny. *J Immunol* 145, 3661-8 (1990)

62. S. D. Gore, M. B. Kastan and C. I. Civin: Normal human bone marrow precursors that express terminal deoxynucleotidyl transferase include T-cell precursors and possible lymphoid stem cells. *Blood* 77, 1681-90 (1991)

63. J. J. van Dongen, H. Hooijkaas, M. Comans-Bitter, K. Hahlen, A. de Klein, G. E. van Zanen, M. B. van't Veer, J. Abels and R. Benner: Human bone marrow cells positive for terminal deoxynucleotidyl transferase (TdT), HLA-DR, and a T cell marker may represent prothymocytes. *J Immunol* 135, 3144-50 (1985)

64. A. Galy, M. Travis, D. Cen and B. Chen: Human T, B, natural killer, and dendritic cells arise from a common bone marrow progenitor cell subset. *Immunity* 3, 459-73 (1995)

65. I. Hoebeke, M. De Smedt, F. Stolz, K. Pike-Overzet, F. J. Staal, J. Plum and G. Leclercq: T-, B- and NK-lymphoid, but not myeloid cells arise from human CD34(+)CD38(-)CD7(+) common lymphoid progenitors expressing lymphoid-specific genes. *Leukemia* 21, 311-9 (2007)

66. D. H. Ryan, B. L. Nuccie, I. Ritterman, J. L. Liesveld, C. N. Abboud and R. A. Insel: Expression of interleukin-7 receptor by lineage-negative human bone marrow progenitors with enhanced lymphoid proliferative potential and B-lineage differentiation capacity. *Blood* 89, 929-40 (1997)

67. E. M. Six, D. Bonhomme, M. Monteiro, K. Beldjord, M. Jurkowska, C. Cordier-Garcia, A. Garrigue, L. Dal Cortivo, B. Rocha, A. Fischer, M. Cavazzana-Calvo and I. Andre-Schmutz: A human postnatal lymphoid progenitor capable of circulating and seeding the thymus. *J Exp Med* 204, 3085-93 (2007)

68. S. Doulatov, F. Notta, K. Eppert, L. T. Nguyen, P. S. Ohashi and J. E. Dick: Revised map of the human progenitor hierarchy shows the origin of macrophages and dendritic cells in early lymphoid development. *Nat Immunol* 11, 585-93 (2010)

69. R. Haddad, P. Guardiola, B. Izac, C. Thibault, J. Radich, A. L. Delezoide, C. Baillou, F. M. Lemoine, J. C. Gluckman, F. Pflumio and B. Canque: Molecular characterization of early human T/NK and B-lymphoid progenitor cells in umbilical cord blood. *Blood* 104, 3918-26 (2004)

70. J. C. Zuniga-Pflucker: T-cell development made simple. *Nat Rev Immunol* 4, 67-72 (2004)

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, $Lineage^-$ $Sca1^+$ $cKit^+$; 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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