Lymphoid priming in human bone marrow begins before expression of CD10 with upregulation of L-selectin

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Expression of the cell-surface antigen CD10 has long been used to define the lymphoid commitment of human cells. Here we report a unique lymphoid-primed population in human bone marrow that was generated from hematopoietic stem cells (HSCs) before onset of the expression of CD10 and commitment to the B cell lineage. We identified this subset by high expression of the homing molecule L-selectin (CD62L). CD10−CD62Lhi progenitors had full lymphoid and monocyctic potential but lacked erythroid potential. Gene-expression profiling placed the CD10−CD62Lhi population at an intermediate stage of differentiation between HSCs and lineage-negative (Lin−) CD34+CD10+ progenitors. CD62L was expressed on immature thymocytes, and its ligands were expressed at the cortico-medullary junction of the thymus, which suggested a possible role for this molecule in homing to the thymus. Our studies identify the earliest stage of lymphoid priming in human bone marrow.

Although much is known about the identity of progenitor stages in mouse lymphopoiesis, considerably less is understood about the critical stages of lymphoid commitment of human hematopoietic cells. Early models developed from mouse studies assumed strictly dichotomous pathways of lineage commitment1. Those ideas have evolved into models of gradual loss of lineage potential that can occur via multiple alternative pathways, although the physiological relevance of lineage potential demonstrated in certain in vitro assays continues to be debated2–5.

A stage at which mouse bone marrow progenitors are 'lymphoid primed' before complete loss of myeloid potential has been defined on the basis of expression of the cell-surface receptor Flt3, and cells at this stage have been called 'lymphoid-primed multipotent progenitors' (LMPPs)2. Critical species-specific differences create challenges for the 'translation' of knowledge about cellular hierarchies derived from mouse studies to the specifics of human hematopoiesis6. In addition, the source and stage in ontogeny of human hematopoiesis can influence the functional abilities, surface immunophenotypes and transcriptional profiles of the cells under study6–8. Most studies of the earliest progenitor stages of human hematopoiesis have used neonatal umbilical cord blood (UCB) as the source of hematopoietic cells. However, understanding how lymphopoiesis is regulated during steady-state adult hematopoiesis requires direct study of hematopoietic stem cells and progenitors from postnatal human bone marrow8,9.

The stepwise process of the lymphoid differentiation of multipotent hematopoietic stem cells (HSCs) in human bone marrow has been assumed to begin with expression of the cell-surface antigen CD10 (CALLA or MME) on CD34+ progenitors, based on the finding that CD10+ progenitors lack myeloid and erythroid potential but are able to generate all lymphoid lineages10. However, subsequent studies have shown that CD34+CD10+ cells, even those without expression of lineage markers (Lin−: CD3−CD14−CD15−CD19−CD56−CD235a−), show a strong bias toward B cell potential with relatively little T cell or natural killer (NK) cell potential11,12. CD34+Lin−CD10+ cells that lack expression of CD24 are precursors of the CD34+Lin−CD10+CD24+ population but nonetheless show molecular evidence of commitment to the B cell lineage, with expression of several B cell–specific genes12.

Therefore, to understand the progenitor hierarchy of the lymphoid commitment of human cells, we sought to identify a stage of lymphoid priming that precedes commitment to the B lymphoid lineage, either before or independently of CD10 expression.

L-selectin (CD62L) is expressed on lymphocytes and mediates homing to peripheral lymphoid organs13. Studies have reported that upregulation of CD62L expression on c-Kit+Lin−Sca-1+ mouse bone marrow cells correlates with loss of erythroid and megakaryocyte potential and efficient thymic engraftment14–16. In this study we have identified a CD34+Lin−CD10− progenitor subpopulation in human bone marrow that had high expression of CD62L and that was devoid of clonogenic myeloid or erythroid potential. In stromal cultures, these cells were able to generate B cells, NK cells and T cells, as well as monocyctic and dendritic cells, similar to the LMPPs in mouse bone marrow that

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have been reported before. CD34+Lin−CD10−CD62Lhi cells (called 'CD10−CD62Lhi cells' here) rapidly engrafted immunodeficient mice, producing B cells and myeloid cells. Despite evidence of lymphoid skewing, comprehensive molecular analysis showed that CD10−CD62Lhi cells not only lacked B cell–specific transcripts but also had not initiated DNA recombination, as determined by their lack of expression of the recombination component–encoding genes RAG1 and RAG2 and minimal expression of DNTT, which encodes terminal deoxynucleotidyltransferase. Genome-wide expression and functional analysis identified the CD10−CD62Lhi progenitor population as a developmental intermediate between the multipotent CD34+Lin−CD38+ human bone marrow cells (2.8% we detected low expression of CD7 on a small population of megakaryocytic-erythroid progenitors and common myeloid progenitors, were readily detectable in the CD34+Lin−CD10−CD7+ population by assay of colony-forming unit cells (CFU-C; Fig. 1b). Consistent with published studies of bone marrow and UCB, CD34+Lin−CD10− progenitors were devoid of clonogenic myeloid or erythroid progenitors (Fig. 1b).

**RESULTS**

**The lymphoid marker CD7 does not define lymphoid commitment**

Given published reports linking expression of the lymphoid marker CD7 to early stages of lymphoid commitment in UCB, we first investigated whether expression of CD7 was sufficient to identify lymphoid commitment in human bone marrow independently of CD10 expression. Examination of CD34+ cell populations depleted of lineage marker–expressing cells showed that the CD34+Lin−CD38+CD7+ population identified before in UCB was not detectable in human bone marrow (Supplementary Fig. 1a). However, as noted before, we detected low expression of CD7 on a small population of CD34+Lin−CD38+ human bone marrow cells (2.8% ± 0.6%; n = 5 donors), most of which did not coexpress CD10 (Fig. 1a). Clonogenic assays demonstrated that CD7 expression alone was insufficient to define lymphoid restriction of the CD34+Lin−CD10− population of bone marrow; nonlymphoid clonogenic cells, particularly erythroid progenitors, were readily detectable in the CD34+Lin−CD10−CD7+ population by assay of colony-forming unit cells (CFU-C; Fig. 1b).

**CD62Lhi progenitors do not have CFU-C potential**

The naive-cell marker CD45RA has been shown to be expressed on various lymphoid progenitors and granulocyte-macrophage progenitors. Analysis of the CD34+Lin−CD10− subpopulation demonstrated the presence of both CD45RA− and CD45RA+ fractions; in contrast, all CD34+Lin−CD10+ cells were CD45RA+ (Fig. 1c). Erythroid potential was absent, but clonogenic myeloid progenitors were still readily detectable, by assay of CFU granulocytes-macrophages, in the CD34+Lin−CD10−CD45RA+ population (Fig. 1d). As expected, clonogenic erythroid potential was high in megakaryocytic-erythroid progenitors and common myeloid progenitors (Fig. 1d), neither of which express CD45RA.

Further refinement of the CD10−CD45RA− population was needed to identify those cells that lacked clonogenic myeloid potential. As noted above, CD62L is a cell-surface receptor that mediates the homing of lymphocytes to peripheral lymph nodes and is expressed on certain mouse bone marrow progenitors that lack erythroid or megakaryocytic potential. Further refinement of the CD10−CD45RA− population demonstrated that although most cells had low expression of CD62L, a distinct subpopulation (9% ± 1.5%; n = 14 donors) of CD34+Lin−CD10−CD45RA− cells in normal human bone marrow had high expression of CD62L (Fig. 1e). Functional screening of CD34+Lin− fractions by assay of CFU-C demonstrated that only the CD34+Lin−CD10−CD62Lhi population (called 'CD10−CD62Lhi cells' here) and the CD34+Lin−CD10− population (called 'CD10− cells' here) were devoid of clonogenic myelo-erythroid potential (Fig. 1f) and Supplementary Table 1). Of note, the CD34+Lin−CD10−CD45RA− population with intermediate expression of CD62L had low but detectable CFU-C potential (population B, Supplementary Fig. 1b), which suggested that progressive loss of multipotency correlated with increasing CD62L expression.
CD10+ cells had low or undetectable expression of CD62L, and the CD34+CD38−/CD10−CD62Lhi population, which is enriched for HSCs and multipotent progenitors (MPPs) [1], had intermediate expression of CD62L. Consistent with published studies [1], CD10+ cells (all of which were CD19+ through depletion of lineage marker–expressing cells) generated mostly B cells with relatively weak potential to develop into NK cells. Cell output under B cell–NK cell lymphoid conditions tended to be higher in cultures initiated with CD10−CD62Lhi cells than in those initiated with CD10+ cells [Fig. 2b]. After in vitro culture under T cell conditions, CD10−CD62Lhi cells generated cells that had the immunophenotype typical of thymocytes (expression of CD1A, CD7, CD4, CD8, CD3 and TCRβ) [2, 3] and that expressed the T cell–associated genes TCF7, GATA3, DNTT and RAG1 [Supplementary Fig. 3], as well as CD56+ NK cells (some of which coexpressed CD8). Cell output was significantly higher in T cell cultures initiated with CD10−CD62Lhi cells than in those initiated with CD10+ cells [Fig. 2e].

Although we did not detect clonogenic myeloid cells by assay of CFU-C, both the CD10+ and CD10−CD62Lhi subsets were able to generate low numbers of myeloid cells when cultured on stromal layers; however, cell output from both progenitor types was significantly lower than that of HSCs-MPPs (Fig. 2f). Most nonlymphoid cells generated from the CD10+ and CD10−CD62Lhi populations in stromal coculture were CD14+CD33+ monocytes–macrophages or CD209+CD1a+ dendritic cells (Supplementary Fig. 4); CD66b+ granulocytes were uncommon. We rarely noted erythroid differentiation in CD10+ or CD10−CD62Lhi cultures, but the production of erythroid cells was robust in cultures from CD38−HSCs-MPPs.

The cloning efficiency of CD10−CD62Lhi cells in lymphoid cultures initiated with a single cell (~11%) and by limiting-dilution analysis (cloning efficiency, 1 cell in 5.3 cells for B cells–NK cells; 95% confidence interval, 4.6–6.4) and 1 cell in 5.6 cells for T cell cultures (95% confidence interval, 4.6–6.9); Fig. 3a,b) was similar to that of CD10+ cells (~12% from single cells). However, lineage analysis of clones demonstrated that the CD10−CD62Lhi population contained bi-potent B cell–NK cell progenitors, whereas the CD10+ population contained predominantly unipotent B cell progenitors (Fig. 3c). We detected myeloid cells in 86% of the clones that could be assigned a lineage in B cell–NK cell conditions (Fig. 3d) and in 97% of all clones assayed from T cell cultures (Fig. 3e). Consistent with the in vitro assays of lineage potential, intrathal transplantation of CD10−CD62Lhi progenitors into immunodeficient mice of the NSG strain (nonobese diabetic–severe combined immunodeficiency strain, deficient in the interleukin 2 (IL-2) receptor IL-2Rγ) produced rapid marrow engraftment of both myeloid and B lymphoid cells (Fig. 3f,g and Supplementary Fig. 5). Differentiation of non–self-renewing progenitors into T lymphoid cells would not be expected in this xenogeneic adult mouse model.

In summary, our functional assays showed that the CD10−CD62Lhi population had full lymphoid potential, was less skewed toward the B lineage than was the CD10+ population, and had greater potential to develop into T cells than did CD10+ population. Although the population lacked clonogenic myelo-erythroid potential, some differentiation of the CD10−CD62Lhi population into myeloid cells...
Figure 3  Lineage potential of CD10−CD62Lhi cells by in vitro clonal analysis and in vivo transplantation studies. (a,b) Limiting-dilution analysis of CD10−CD62Lhi cells grown in B cell–NK cell conditions (a) or T cell conditions (b), presented as frequency of wells lacking B cells–NK cells (B-NK− wells) or T cells (T− wells). (c) Lineage analysis of clones from single CD10−CD62Lhi or CD10+ cells in B cell–NK cell lymphoid coculture, presented as frequency of wells with clonal growth containing NK cells (NK), B cells (B) or both (B & NK). (d) Flow cytometry of clones generated in B cell–NK cell conditions from one to three CD10−CD62Lhi cells showing NK cell potential (CD56+), myeloid potential (CD14+CD15+) and dendritic cell potential (CD1a+) of one clone (far left and middle left); B cell potential (CD19+) and dendritic cell potential (CD1a+; middle right); or coexpression of myeloid and dendritic cell markers from a single-cell clone (far right). (e) Flow cytometry of a single clone generated in T cell conditions showing T cell potential (CD4+CD8+) and myeloid potential (CD14+CD15+) and dendritic cell potential (CD1a+) of one clone (far left and middle left); B cell potential (CD19+) and dendritic cell potential (CD1a+; middle right); or coexpression of myeloid and dendritic cell markers from a single-cell clone (far right). (f) Flow cytometry of bone marrow from a mouse of the NSG strain, analyzed 2 weeks after transplantation of 1 × 10^5 irradiated CD34− carrier cells only (negative control; left) or 3 × 10^4 CD34+Lin−CD10−CD62Lhi cells (center) or 1.5 × 10^5 CD34−Lin−CD10−CD62Lhi cells by analysis of CD10−CD62Lhi cells grown in vitro–in vivo, and back-gating of B cells and myeloid cells from plots above (bottom row). SSC, side scatter; FSC, forward scatter. (g) Frequency of human myeloid cells and human B cells among the total human cells in back-gating of B cells and myeloid cells from plots above (bottom row). SSC, side scatter; FSC, forward scatter. (h) Frequency of human myeloid cells and human B cells among the total human cells in clonal analysis and back-gating of B cells and myeloid cells from plots above (bottom row). SSC, side scatter; FSC, forward scatter. (i) Frequency of human myeloid cells and human B cells among the total human cells in clonal analysis and back-gating of B cells and myeloid cells from plots above (bottom row). SSC, side scatter; FSC, forward scatter. (j) Frequency of human myeloid cells and human B cells among the total human cells in clonal analysis and back-gating of B cells and myeloid cells from plots above (bottom row). SSC, side scatter; FSC, forward scatter. (k) Frequency of human myeloid cells and human B cells among the total human cells in clonal analysis and back-gating of B cells and myeloid cells from plots above (bottom row). SSC, side scatter; FSC, forward scatter. 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Figure 4  CD10−CD62Lhi cells represent an intermediate stage of differentiation between HSCs and CD10+ progenitors. (a) Expression of cell-surface markers on various CD34−Lin− populations (key), assessed by flow cytometry (top row, left; bottom row), and summary of those results (top right), presented as mean fluorescence intensity (MFI). *P < 0.010 and **P < 0.001 (t-test). (b) Flow cytometry of B cell–NK cell lymphoid cultures initiated with CD38−, CD10−CD62Lhi or CD10+ cells and assessed at 1 week. (c) Unsupervised whole-genome principal-component analysis of CD38−, CD10−CD62Lhi or CD10+ populations from human bone marrow. Data are from eighteen experiments with two or three independent samples per marker (or fourteen samples for CD38; a), two independent experiments (b) or three experiments with three independent samples (c).

(mostly monocytes-macrophages and dendritic cells) was induced in stromal cocultures and in short-term engraftment assays. However, its myeloid potential was significantly lower than that of HSCs-MPPs, and erythroid potential was absent.

Differentiation stages of HSCs and lymphoid progenitors

Given the lineage potential shown in the functional studies reported above, we next explored the stages of differentiation of the CD10−CD62Lhi and CD10+ populations compared with those of the...
most primitive CD34^+Lin^−CD38^− HSC-MPP population (called 'CD38^− cells' here). Expression of the differentiation marker CD38 increased progressively from the CD34^+CD38^− population to the CD10^+CD62L^hi population and was maximal in the CD10^+ population (n = 14 donors; Fig. 4a). Expression of the stem cell–associated receptors c-Kit, Flt3, integrin α6 (CD49F) and Prom-1 (CD133) was similar in CD38^− and CD10^−CD62L^hi populations but was downregulated in CD10^+ cells; Thy-1 (CD90) had its highest expression on CD38^− cells. HLA-DR was upregulated in both CD10^−CD62L^hi and CD10^+ progenitors (Fig. 4a). After 1 week in lymphoid culture, CD10^−CD62L^hi populations differentiated and lost expression of the adhesion molecule CD34 faster than did CD10^+CD62L^hi cells (Fig. 4b). In addition, CD10^+CD62L^hi cells were able to generate CD34^+CD10^+ cells in vitro (Fig. 4b), which suggested that CD10^+CD62L^hi cells were precursors of the CD10^+ population.

Principal-component analysis of global gene-expression data from microarray analysis of three different bone marrow samples also placed the CD10^−CD62L^hi progenitors in an intermediate position between the CD38^− HSC-MPPs and the CD10^+ progenitors (Fig. 4c). Gene expression of CD10^−CD62L^hi progenitors clustered hierarchically with CD38^− HSC-MPPs rather than with CD10^+ progenitors (Supplementary Fig. 6a). By pairwise comparison with HSCs-MPPs, similar numbers of genes were upregulated in CD10^−CD62L^hi and CD10^+ populations; approximately half of those upregulated genes were common to both progenitor types (Supplementary Fig. 6b). More than twice as many genes were downregulated in the CD10^+ population than were downregulated in the CD10^−CD62L^hi population, and most downregulated genes in CD10^−CD62L^hi cells were also downregulated in CD10^+ cells (Supplementary Fig. 6b). Thus, the differentiation of HSCs-MPPs involved many shared molecular pathways, but additional transcriptional modulation seemed to occur after the CD10^−CD62L^hi stage during the generation of CD10^+ cells.

**Downregulation of HSC-associated genes in CD10^−CD62L^hi cells**

We then analyzed by microarray and quantitative PCR the expression patterns of genes encoding molecules known to regulate critical hematopoietic stages of differentiation to delineate the molecular

![Figure 5](image_url)

**Figure 5** CD10^−CD62L^hi cells represent a distinct progenitor population with a unique expression profile that combines genes of HSCs and early lymphoid cells. (a) Expression of genes encoding transcription factors (a) or cytoplasmic and cell-surface molecules (b) with a difference in expression of more than twofold by pairwise comparison (P < 0.05) and defined as follows based on statistical analysis (not heat-map appearance): cluster 1, upregulated only in CD38^− cells relative to expression in the two other equivalent populations (CD38^− > CD10^−CD62L^hi = CD10^+); cluster 2, CD38^− > CD62L^hi > CD10^+; cluster 3, (CD38^− = CD10^−CD62L^hi) > CD10^+; cluster 4, CD10^−CD62L^hi > CD38^− = CD10^+; cluster 5, CD10^− > CD38^− = CD10^+; cluster 6, CD10^+ > CD10^−CD62L^hi > CD38^−). (c) Quantitative PCR analysis of gene expression, presented relative to expression in CD10^−CD62L^hi cells. *P < 0.05, **P < 0.01 and ***P < 0.001 (one-way ANOVA). (d) Quantitative PCR analysis of gene expression in single cells, presented as the frequency of single cells expressing the gene. Data are representative of three independent experiments (a,b), three experiments with three biological replicates (c; mean and s.e.m.) or one experiment with 13 cells assayed per gene (d).
relationships among the CD38⁻, CD10⁻CD62Lhi and CD10⁺ populations. All genes included in the heat maps had a difference in expression of at least twofold in one population relative to their expression in the other two populations (P < 0.05 (moderated t-statistics)) and belonged to one of six different expression patterns (clusters 1–6; Fig. 5a,b). Genes encoding known HSC-related transcription factors (TAL1, GATA2 and PRDM16) were significantly downregulated in both CD10⁻CD62Lhi cells and CD10⁺ cells relative to their expression in the CD38⁺ population (cluster 1; Fig. 5a). Genes of the HOXB family were also downregulated during the transition from the CD38⁻ HSC-MPP stage to the CD10⁻CD62Lhi LMPP stage with no further significant change at the CD10⁺ stage (cluster 1; Fig. 5a). In contrast, expression of genes of the HOXA family decreased later in differentiation at the CD10⁺ progenitor stage (clusters 2 and 3; Fig. 5a). We noted reciprocal patterns of expression for members of the polycomb repressive complexes PRC1 (encoded by PGC2, PHC2, and SCML4) and PRC2 (encoded by SUZ12, EZH2 and EED)²⁴ (clusters 1 and 5, respectively; Fig. 5a). These analyses identified a highly coordinated program of transcriptional regulation as HSCs lost multipotency, became lymphoid primed and then committed to B lymphopoiesis.

Lymphoid-differentiation stages of CD10⁻CD62Lhi and CD10⁺ cells

Analysis of genes upregulated only in the CD10⁻CD62Lhi population (cluster 4; Fig. 5b) showed a profile consistent with the dual lymphoid and monocyte potential of this population. Specifically, genes associated with T cell and NK cell lineages (CD2 and CD3E)¹²,¹⁵–²⁷ and genes encoding lymphoid cytokine receptors (IL2RG, IL10RA, IL10RB, IL17RA and IFNGR1) were upregulated, as were myeloid cell–associated genes (MPO, CSF1R and CSF2R; Fig. 5b). Consistent with the cell surface expression of Flt3, FLT3 was expressed in both HSCs-MPPs and CD10⁻CD62Lhi cells but not in CD10⁺ cells (cluster 3; Fig. 5b). Consistent with the B cell–skewed differentiation potential of the CD10⁺ population, genes known to be expressed specifically during commitment to the B cell lineage (EBF1, PAX5, IL7R, CD79A, CD79B, VPREB1, VPREB3, CD19, CD22, CD24 and CD27) had high expression in CD10⁺ cells (cluster 6; Fig. 5a–c). Notably, none of those B cell–specific genes were expressed in either CD34⁺CD38⁻ cells or CD10⁻CD62Lhi cells.

Detailed analysis by quantitative PCR showed that although expression of genes encoding molecules essential for lymphoid commitment was highest in CD10⁺ cells, upregulation of certain genes encoding molecules of early lymphoid differentiation began at the CD10⁻CD62Lhi stage. Expression of TCF3 (which encodes the transcription factor E2A) increased 2.1-fold during the transition from CD38⁻ cell to CD10⁻CD62Lhi cell and increased 4.4-fold in the transition from CD10⁻CD62Lhi cell to CD10⁺ cell (Fig. 5c). Similarly, DNTT expression increased 8.0-fold during the transition from CD38⁻ cell to CD10⁻CD62Lhi cell and increased 12.0-fold in the transition from CD10⁻CD62Lhi cell to CD10⁺ cell (Fig. 5c). In contrast, RAG1 expression was limited to CD10⁺ cells (Fig. 5c), which demonstrated that the mechanisms of DNA rearrangement for genes encoding T cell antigen receptors and immunoglobulins were not fully initiated in the CD10⁻CD62Lhi population.

To investigate further the degree of heterogeneity of the three populations, we assayed the expression of key genes in single cells (Fig. 5d). These analyses showed that the HSC genes TAL1 (which encodes the transcription factor SCL (Tal-1)) and MPL (which encodes the thrombopoietin receptor) were expressed exclusively in CD38⁻ cells, and expression of RAG1 and PAX5 (which encodes the transcription factor Pax5) was limited to CD10⁺ cells. We detected expression of TCF3 in a similar percentage of CD10⁺CD62Lhi and CD10⁺ cells. Detectable FLT3 expression in single cells was limited almost exclusively to the CD10⁻CD62Lhi population (Fig. 5d). Thus, the CD38⁻ HSC-MPP, CD10⁻CD62Lhi and CD10⁺ populations had distinct molecular profiles, consistent with their functional ‘readout’ in vitro. Whereas the CD10⁺ population was committed to B lymphopoiesis, the CD10⁻CD62Lhi population included cells with evidence of...
early lymphoid priming but no expression of genes associated with commitment to the B cell lineage (Supplementary Fig. 7).

**CD62L and ligand expression in human thymus**

We analyzed by flow cytometry and gene expression the coexpression of receptor-ligand pairs that have been reported before in mouse studies as being important in the homing of cells to and settling of cells in the thymus. Expression of the chemokine receptor CXCR4 was similar in CD10+CD62Lhi and CD10+ populations (Fig. 6a). However, PSLG-1 (the ligand for P-selectin) and the activation and memory marker CD44 had higher expression in CD10+CD62Lhi cells than in CD10+ cells (Fig. 6a). In addition, the gene encoding the chemokine receptor CCR7, which is expressed on mouse early thymic progenitors and mediates the migration of early thymocytes, was significantly upregulated in the CD10+CD62Lhi population relative to its expression in either CD10+ cells or the CD38+ population (Fig. 6b). We noted no consistent differences between the populations in their expression of the chemokine CCR9 (data not shown).

We next examined the expression of CD62L in progenitor populations from human thymus. CD62L expression was higher in CD34+ thymic progenitors than in the more mature CD34+ thymocytes (which represent >95% of all thymocytes; Fig. 6c). After further fractionation of the CD34+ thymocyte population, we found that most CD62L-expressing cells were in the CD34+CD1a- subset rather than the more mature CD34+CD1a+ subset (Fig. 6c). MECA-79 detects a carbohydrate epitope found on the family of CD62L ligands known as ‘peripheral node addressins’. We detected staining of MECA-79 in the thymic vasculature specifically in a subset of P-selectin-positive endothelial cells at the cortico-medullary junction of the thymus, the site of entry of marrow-derived precursors into the thymus (Fig. 6d–j); this suggested a possible role for CD62L in homing to the human thymus.

**DISCUSSION**

Our studies presented here have demonstrated that ‘lymphoid priming’ in human bone marrow began before the onset of CD10 expression in a subset of CD34+ progenitors with high expression of the homing molecule CD62L. Several pieces of evidence suggested that the CD10+CD62Lhi population was a precursor of the more B cell–restricted CD10+ stage of lymphopoiesis. First, it is widely assumed that all human B cell differentiation passes through a CD10+ progenitor stage, and cultures initiated with CD10+CD62Lhi cells were able to generate CD10+ progenitors before differentiating into CD19+ B cells. In addition, although the CD10+CD62Lhi population had greater NK cell potential, the number of B cells generated in culture was at least equivalent to that generated in cultures of CD10+ cells. The patterns of the expression of genes and cell-surface antigens were also consistent with a model that positions the CD10+CD62Lhi population before CD10 expression.

A published study has described a CD10+ subset in a CD34+CD38neg–lo population with lymphoid, monocytic and dendritic cell potential but no erythroid potential. However, this CD10+ ‘multilymphoid progenitor’ also expressed the B cell–specific gene PAX5. Notably, the strategy for the isolation of those multilymphoid progenitors included cells with intermediate expression of CD38, similar to expression in the CD10+CD62Lhi population and higher than that in the most primitive HSC fraction. We propose that lymphoid priming begins with upregulation of CD38 (relative to its expression in HSCs) and that B cell commitment is initiated with the onset of CD10 expression and further upregulation of CD38 expression.

Most human hematopoietic studies have used UCB, largely because this source of human cells is more readily accessible than is bone marrow. The proliferative output of bone marrow progenitors is much lower than that of their immunophenotypic homologs in UCB or that of HSCs from either source, which makes in vivo assessment of rare, non–self-renewing bone marrow progenitor populations difficult and sometimes unfeasible. However, UCB does not represent steady-state postnatal hematopoiesis, and substantial differences between progenitors from UCB and bone marrow are known to exist for both immunophenotype and function. Notably, the functional and molecular profiles of CD10+CD62Lhi bone marrow progenitors (which do not express CD7) are similar to those of CD34+CD38+CD7+ UCB progenitors. Moreover, we found that the CD10+CD62Lhi immunophenotype described here was less reliable for the identification of a pure lymphoid-primed population in UCB than in bone marrow; a distinct CD62Lhi population was difficult to detect in UCB, and CD34+Lin+CD10+CD45RA+CD62L+ cell populations in UCB included small but readily detectable numbers of CFU-C (Q.-L.H. and G.M.C., unpublished data). The differences in the lineage potentials of cells with similar immunophenotypic differences in UCB and bone marrow, as well as the intrinsic functional differences that would be expected between cells that are detected transiently in the postnatal circulation and those that are generated throughout life in the bone marrow microenvironment, highlight the critical need for studies that focus on human bone marrow.

Considerable data have been generated in mouse studies to both support and challenge the classic idea that the lymphoid and myeloid-erythroid pathways emerge separately from a multipotent progenitor stage. The ‘lymphoid-primed’ LMPPs in mouse bone marrow retained full lymphoid and some myeloid potential but had lost erythroid-megakaryocytic potential, whereas common lymphoid progenitors (CLPs) represented a more mature, lymphoid-restricted progenitor population. Cell surface expression of Flt3 has been used to isolate LMPPs from a subpopulation of c-Kit+Lin- Sca-1+ cells in mouse bone marrow, and the IL-7 receptor IL-7Rα is used to define mouse CLPs in the c-Kit+Lin- Sca-1+ population. Given our functional and molecular data, the CD10+CD62Lhi human bone marrow progenitor seems most similar to the mouse LMPPs, and the CD10+ progenitor is more analogous to the mouse CLPs. However, despite upregulation of FLt3 at the transcriptional level, cell-surface expression of Flt3 has not been found to be useful as a marker for discriminating between human HSCs and LMPPs (and reported here). Notably, studies have reported that upregulation of CD62L expression in c-Kit+Lin- Sca-1+ mouse bone marrow cells correlates with high expression of Flt3 and loss of erythroid and megakaryocyte potential, which suggests that CD62L expression might be used as an alternative marker for discriminating between mouse multipotent progenitors and LMPPs.

The myeloid output of the CD10+CD62Lhi population consisted mostly of monocyte-macrophage and dendritic cells. The absence of clonogenic myeloid-erythroid potential in assays of CFU-C suggested that the CD10+CD62Lhi population does not represent a precursor of the main myeloid-erythroid pathways initiated by common myeloid progenitors and granulocyte-macrophage progenitors. Instead, we favor the proposal that the CD10+CD62Lhi cells are ‘lymphoid-primed’ progenitors that precede CD10 expression and are able to generate limited numbers of monocytes-macrophages and dendritic cells. This type of residual myeloid and dendritic potential has been reported for even more lymphoid-committed progenitors. A published paper has noted that mouse IL-7Rα+ CLPs, despite their complete lack of either CFU activity or in vivo myeloid potential,
can generate myeloid cells in stromal cocultures, which suggests that myeloid differentiation may be an alternative pathway in certain in vitro conditions. Nonetheless, it is clear that the capacity for myeloid differentiation in vitro progressively wanes as lymphoid commitment proceeds and that this residual, mostly monocytic potential is retained after erythroid potential is lost.

We noted both differences and similarities between the CD10−CD62Lhi cells and mouse LMPPs in their gene expression. In both mouse LMPPs and human CD10−CD62Lhi cells, genes encoding the transcription factor Tal-1 and the cytokine receptor MPL are substantially downregulated relative to their expression in HSCs, whereas expression of the gene encoding c-Kit is retained. Expression of the gene encoding E2A, which is essential for the development of mouse LMPPs, was also upregulated during generation of the CD10−CD62Lhi population from HSCs-MPPs, but B cell–specific genes such as EBF1 and PAI5 were not. In contrast, components of the molecular machinery required for DNA recombination seem to have high expression in mouse LMPPs, but in our human studies, RAG1 and RAG2 were expressed at the CD10+ stage and DNTT expression was significantly higher in CD10+ cells than in CD10−CD62Lhi cells.

The identification of a lymphoid-primed progenitor that may be a precursor of the CD10−CLIP raises the question of whether CD10−CD62Lhi cells are recruited to the thymus to initiate T cell differentiation. Controversy about the identity of precursors that seed the mouse thymus has continued for over a decade, and it seems likely that more than one type of bone marrow progenitor may be able to initiate thymopoiesis. Experimental restrictions make it impossible to definitively prove the identity of the bone marrow precursors that normally seed the human thymus. The CD10−CD24− population in bone marrow possibly represents a lymphoid progenitor that seeds the human thymus, given the finding of a similar immunophenotypic subset among human thymocytes. The thymocyte data presented here have provided evidence that CD10−CD62Lhi cells may be an additional or alternative thymic precursor population. It should be noted that although CD62L expression was highest on CD34+CD1a+ progenitors, CD10−CD62Lhi bone marrow cells are not precursors of the most primitive (CD7−) subset of CD34+CD1a− thymocytes. CD34+CD1a−CD7− thymocytes have high myeloid and erythroid potential in clonal assays and do not express CD62L. It is not clear at this time whether CD62L becomes upregulated as CD34+CD1a−CD7− MPPs differentiate into CD34+CD1a+CD7− thymocytes or whether CD7 is rapidly upregulated when CD7−CD62Lhi LMPPs engage with the thymic microenvironment. PSGL-1–P-selectin interactions are critical mediators of homing to mouse thymus. As PSGL-1 had abundant expression on both HSCs-MPPs and CD10−CD62Lhi bone marrow cells, it is possible that homing to human thymus involves the same mechanism. However, the high expression of CD62L in the primitive CD34+CD1a+ thymocyte population and the endothelial expression of CD62L ligands in the human thymus, specifically in the corticomedullary region, raise the possibility that in addition to being involved in the homing of lymphocytes to peripheral lymphoid organs, CD62L may have a role in the homing of progenitors to human thymus. We have noted expression of CD62L in a subset of CD34+Lin−CD10− cells (but not CD34+CD10− cells) in mobilized peripheral blood (data not shown), but the physiological relevance and lineage potential of this mobilized population is as yet unclear. Of note, although interactions with CD62L have not been described in homing to mouse thymus, CD62L expression has been used to identify a population of mouse bone marrow progenitors that efficiently and rapidly reconstitute the mouse thymus after transplantation and a population of c-Kit+Lin−Sca-1+CD62L− RAG-1− progenitors is present in both mouse bone marrow and mouse thymus.

The reliance on CD10 expression as a marker of lymphoid commitment in previous studies of hematopoietic progenitors in human bone marrow has until now meant that states of differentiation could be compared only between multipotent progenitors and progenitors committed to the B cell lineage. The identification of a progenitor in human bone marrow primed for full lymphoid differentiation, and at a stage before B cell commitment, will now permit delineation of the molecular regulation of the first stages of lymphoid commitment in human hematopoiesis. It will also allow understanding of how these processes are affected during aberrant hematopoiesis in disease states.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. GEO: microarray data, GSE35685.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

L.A.K. designed, did and analyzed experiments and wrote the paper; Q.-L.H. designed, did and analyzed experiments, R.S. did bioinformatics analysis of microarray data; S.G. and Y.Z. assisted in experiments; C.P. did experiments; L.A.K. designed, did and analyzed experiments and wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Isolation of bone marrow cells. Normal human bone marrow and thymic cells were obtained from healthy donors via the Pathology Tissue Core of the University of California, Los Angeles, Cincinnati Children's Hospital, or AllCells according to guidelines approved by Institutional Review Board of the University of California, Los Angeles. Samples were enriched for CD34+ cells by the magnetic-activated cell-sorting (MACS) system (Miltenyi Biotec).

Samples enriched for CD34+ cells were incubated with combinations of monoclonal antibodies specific for human molecules (allophycocyanin–indotricarbocyanine–conjugated antibody to anti-CD34; 581; Biolegend); and phycoerythrin–indodicarbocyanine–anti-CD5RA (HI100), allophycocyanin–anti-CD38 (HIT2), phycoerythrin–indodicarbocyanine–anti-CD10 (H110a), phycoerythrin–anti-CD62L (DREG-56), phycoerythrin–or phycoerythrin–indodicarbocyanine–anti-CD7 (M-T701), and fluorescein isothiocyanate–labeled lineage-depletion antibodies anti-CD3 (SK7), anti-CD14 (M2E2), anti-CD19 (4G7), anti-CD56 (MY31) and anti-CD253a (GA-R2; all from Becton Dickinson). The DNA-intercalating dye DAPI (4′,6-diamidino-2-phenylindole) was added for analysis of viability. A ‘no-antibody’ control defined negative gates. Additional analyses used the following antibodies: Alexa Fluor 647–anti-CD127 (HIL-7R-M21), allophycocyanin–anti-CD117 (YB5.88), allophycocyanin–anti-CD184 (anti-CXCR4; 12G2), allopheocyanin–anti-PSGL-1 (anti-CD162 or anti-SELPLG; KPL-1), phycoerythrin–anti-Flt3 (anti-CD135; 4G8), allophycocyanin–anti-CD44 (G44.26), allophycocyanin–anti-CD62L (DREG-56), phycoerythrin–indodicarbocyanine–anti-CD90 (5E10) and phycoerythrin–anti-HLA-DR (L234; all from BD). Cells were isolated on a FACSAria (355-, 405-, 488-, 561- and 633-nm lasers; BD Immunocytometry Systems).

B cell–NK cell lymphoid cultures. Cells isolated by flow cytometry were plated in bulk on OP9 stroma in 48-well plates or as single cells or by limiting dilution on OP9 or MS5 stroma in 96-well plates with an automated cell-deposition unit. Cells were cultured in lymphoid medium (RPMI-1640 medium (Irvin Scientific) with 5% FCS (Biowhittaker), 50 µM 2-mercaptoethanol (Sigma), penicillin-streptomycin, 1-glutamine (Gemini Bio Products)) in the presence of IL-7 (5 ng/ml), Flt3 ligand (5 ng/ml) and thrombopoietin (5 ng/ml), with or without IL-3 (5 ng/ml), for first 3–5 d of culture (cytokines were replaced every 48 h). Cells were cultured, then were stained with the following human-specific monoclonal antibodies (alls from BD): anti-CD45 (HI30; for all human hematopoietic cells); anti-CD66B (G10F5; for granulocytic cells). A Fortessa or Aria II (BD) was used for flow cytometry, and data were analyzed with FlowJo software. T cell differentiation was assessed by RT-PCR of human CD45 transcripts.

Microarray analysis. RNA from bone marrow from three different donors was extracted with Microtik (Qiagen) and hybridized onto Affymetrix U133 Plus 2.0 Array (Affymetrix). The robust multichip average method was used to obtain normalized expression from the three populations. The Microarray Suite 5 (MAS5) algorithm was used for present, marginal or absent ‘calls’ for all replicates. Replicate arrays from the three populations were hierarchically clustered with Sørensen rank correlation (distance metric) and average linkage (agglomeration) method. Only probe sets considered ‘present’ by the MAS5 method in all replicates in any of the three populations (24,067 probes) were used for hierarchical clustering. The number of genes with a difference in expression in Venn diagrams was calculated with the R/Bioconductor software package Limma at a P value of less than 0.01 and change in threshold of ± twofold. For genes with multiple probe sets, the probe set with the lowest P value was chosen. Probe sets not mapped to a gene with an official symbol were excluded. Genes were considered for inclusion in the heat map only if they had a difference in expression of ± twofold and the difference was significant at a P value of less than 0.05 relative to expression by the other population of cells in at least one condition (Limma). Gene set enrichment analysis was done as described. For presentation, Cluster 3.0 software (clustering) and Java TreeView software (dendrograms and heat maps) were used.

Immunohistochemistry. Human thymuses were frozen at −80 °C and embedded in optimum cutting temperature compound (Tissue-Tek) and sections 5 µm in thickness were stained with hematoxylin and eosin. For immunohistochemistry, sections were fixed in 10% neutral buffered formalin, then were incubated with primary antibody (anti-MECA-79 (1:83 dilution; sc-19602; Santa Cruz Biotechnology) and/or anti-VE-cadherin (1:83 dilution; BV6; Chemicon International)), followed by incubation with horseradish peroxidase–conjugated secondary antibody (anti-rat (MP-7444; Vector Labs) or anti-mouse (T20912; Invitrogen). For fluorescence immunohistochemistry, Alexa Fluor 594– and/or Alexa Fluor 488– labeled tyramide was applied (for tyramide signal amplification; T20925 and T20912; Invitrogen Molecular Probes). For chromagen staining, DAB (3,3-diaminobenzidine tetrahydrochloride) was applied, followed by hematoxylin (Jackson ImmunoResearch). Sections were viewed with Axioimager Software v3.0.2 (Fluidigm) at the GeneSeq Core of the University of California, Los Angeles. The gene encoding β2-microglobulin was used as a positive control for presence of CDNA.

Quantitative PCR analysis. After isolation of cells from a FACS Aria, RNA was extracted with a Qiagen RNeasy Microkit (Qiagen) and reverse-transcribed with Omniscript RT, Oligo DT, and Rnaguard (Pharmaica Biotech). An ABI 7500 was used for real-time PCR with Taqman MasterMix and TaqMan Probe–based gene-expression analysis (probes (Applied Biosystems), Supplementary Table 3). Reactions were done in technical and biological triplicates. Nine candidate reference genes were analyzed with geNormPlus software for optimal reference genes. Quantitative PCR results were normalized to the geometric means of results obtained for the reference genes ACTB and B2M through the use of the change-in-cycling-threshold methods (ΔΔCt).

T cell lymphoid cultures. Cells were plated in bulk on OP9 stroma in 48-well plates or as single cells or by limiting dilution with OP9 or MS5 stroma in 96-well plates with an automated cell-deposition unit. Cells were cultured in lymphoid medium (RPMI-1640 medium (Irvin Scientific) with 5% FCS (Biowhittaker), 50 µM 2-mercaptoethanol (Sigma), penicillin-streptomycin, 1-glutamine (Gemini Bio Products)) in the presence of IL-7 (5 ng/ml), Flt3 ligand (5 ng/ml) and thrombopoietin (5 ng/ml), with or without IL-3 (5 ng/ml), for first 3–5 d of culture (cytokines were replaced every 48 h). Cells were cultured, then were stained with the following human-specific monoclonal antibodies (alls from BD): anti-CD45 (HI30; for all human hematopoietic cells); anti-CD66B (G10F5; for granulocytic cells). A Fortessa or Aria II (BD) was used for flow cytometry, and data were analyzed with FlowJo software. T cell differentiation was assessed by RT-PCR of human CD45 transcripts.

Statistical analysis. Prism version 5 (GraphPad Software Inc) was used for statistical analysis. The two-way analysis of variance was used for comparison of growth potential. The mean and s.e.m. were calculated for total CFU output of populations, mean fluorescence intensity and quantitative PCR, and the
one-way analysis of variance with a Tukey post-test was used for statistical analysis. ELDA software\textsuperscript{50} was used for limiting dilution analysis.

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