Published in final edited form as:

Curr Opin Hematol. 2013 July ; 20(4): 265–272. doi:10.1097/MOH.0b013e3283612628.

Early Events in Lymphopoiesis, an Update

Qingzhao Zhang¹, Ryuji Iida¹, Takafumi Yokota², and Paul W. Kincade^{1,*}

¹Immunobiology and Cancer Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA

²Department of Hematology and Oncology, Osaka University Graduate School of Medicine, Osaka, Japan

Recent Findings & Summary

We can now appreciate that new lymphoid progenitors are drawn from a heterogeneous collection of hematopoietic stem cells (HSC) through asynchronous patterns of gene expression. Complex interactions then occur between the gene products. While transcription factors have long been a focus of investigation, micro RNAs are also being implicated in lymphopoiesis. Lymphocytes are normally replaced in correct proportion to other blood cells, but ratios change dramatically during infections. Long standing issues relating to T versus B lineage divergence remain but have been enriched with remarkable new findings about thymus seeding. Finally, knowledge obtained from studies of mice is slowly being extended to humans.

Keywords

Hematopoietic cell; B lymphopoiesis; Infection

Introduction

Mechanisms responsible for immune system replenishment have been studied for some time but not with the current level of success. We and others have attempted to capture the essence of that progress, but reviews are incomplete soon after publication^{1–4}. Our last contribution to this series focused on the heterogeneity of hematopoietic stem cells (HSC) as well as developmental relationships to equally diverse progenitors in the B lineage lymphoid series¹. (Figure 1, reproduced with permission) Since then, notable progress has been made in understanding requisite transcription factors and micro RNAs needed for sustaining T lymphocyte production and steps in NK cell formation. Molecular mechanisms are being sought that would account for age related declines in the process as well as infection related changes. There has also been success in identification of lymphoid progenitors in humans. This brief review will try to capture some of those developments, emphasizing B lymphopoiesis as representative of all immune system components.

Transcriptional requirements for lymphopoiesis

Our understanding has increased regarding how early stages of lymphopoiesis are regulated⁵. As one example, Ikaros (Ikzf) family transcription factors are important for early lymphoid priming in HSC, and half of all genes upregulated at the common lymphoid

*To whom correspondence should be addressed: Immunobiology and Cancer Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA Tel: (405) 271-7905, Fax: (405) 271-8568, kincade@omrf.org.

Conflicts of Interest The authors have no conflicts of interest.

progenitors (CLPs) to Fr.A transition are targeted by Ikaros⁶. PU.1 (Sfpi.1) regulates myeloid/lymphoid lineage differentiation in a dose-dependent manner. Low levels of PU.1 favor B lineage fates while high concentrations promote myeloid differentiation. Gfi1 inhibits PU.1 expression in MPP, thereby inducing B lymphopoiesis indirectly⁷. Ikaros then antagonizes PU.1 by upregulating Gfi1.

E2A proteins also play critical roles in early lymphoid specification. A recent study indicates that E2A and HEB function in concert to upregulate the expression of FOXO-1 in CLPs². E2A is likely to bind directly to regulatory elements in the FOXO-1 locus. Deficiency of either E2A or HEB generated similar phenotypes and gene profiles. In both cases, B cell development was arrested at the Ly6D⁺CLP stage.

Sequential activation of E2A, Ebf1, Pax5 and FOXO1 is essential for B-lineage differentiation and commitment. However, these transcription factors work in a cross-regulatory network rather than a hierarchical one-way direction. Chromatin immunoprecipitation (ChIP) sequencing studies found coordinated DNA occupancy and interactions between these key transcriptional regulators⁸. Ebf1 and FOXO1 promote B lineage differentiation in a feed-forward manner⁸. An epigenetic study reported a spectrum of genes that switched nuclear location during early B cell development⁹. That is, E2A, PU.1, Ebf1 and Pax5 seem to bind 'collaboratively' while regulating B lineage differentiation. On the other hand, the locus for the E2A inhibitor Ebf1 was sequestered at the nuclear lamina in MPP to prevent its premature activation.

Ebf-1 and Pax5 are both expressed in CLP subsets¹ and largely upregulated in pro-B cells. They have distinct roles in B cell differentiation and commitment. Pax5-regulated genes in pro-B cells account for about a quarter of all expression changes when cells proceed from CLP to pro-B cell stages¹⁰. Sigvardsson and colleagues crossed λ 5 reporter mice and Pax5 deficient mice¹¹. Surprisingly, λ 5 expressing cells were not reduced in Pax5 null mice compared to WT mice. Results of single cell PCR analysis revealed that Ebf-1, but not Pax5 was required for the expression of B lineage specific genes. Pax5, instead, was essential for inhibiting NK and T cell lineage genes to enable B lineage restriction. This effect of Pax5 is dose dependent¹². Progenitor cells with low levels of Pax5 developed a hybrid B-lymphoid/myeloid phenotype; B220⁺CD19⁺CD11b⁺Gr-1⁺. FOXO family members enhance the expression of IL7-receptors, by which signaling results in activation of FOXO1, suggesting a positive feedback loop in their action for B-lineage determination. Since FOXO1 is a direct target of Ebf1 and E2A, the family might act downstream of the two indispensable factors for the B lineage^{13,14}.

Additional transcription factors have been added to the network. C-Myc regulates cell proliferation, survival and more importantly, expression of Ebf-1¹⁵. C-Myb appears to act upstream of Ebf1 and regulate survival and differentiation of pro-B cells. While c-Myb regulates the expression of IL-7 receptors, the molecule affects pro-B cell survival independent of IL7-receptor signaling^{16,17}. Runx1 is indispensable for the development of authentic HSC responsible for definitive hematopoiesis. Loss of Runx1 or its binding partner Cbfb caused a developmental block in early B-lineage differentiation¹⁸. Expression of E2A, Ebf and Pax5 was reduced in the Runx1-deficient progenitors. Runx1-Cbfb complexes were found to bind the Ebf1 promoter, and Runx1 deletion caused excessive H3K27 trimethylation, that usually suppresses transcription, in the Ebf1 promoter region. ATP11C is a P4-type ATPase. Mice with ATP11C deficiency had diminished follicular B cells. Available data suggests ATP11C is responsible for maintaining progenitors' responsiveness to IL-7 and the level of Ebf-1¹⁹. Bcl11a deficiency selectively reduced HSCs, all lymphocytes and their progenitors²⁰. This is partially due to the anti-apoptotic activity of

Bcl11a. It is also required for lymphopoiesis from HSCs and LMPPs via mechanisms that do not involve Ikaros, E2A or Ebf-1.

Roles for microRNAs

Many microRNAs (miRNAs) are expressed in HSC and progenitor cells (Figure 2). They are important post-transcriptional regulators of gene expression that inhibit messenger RNA (mRNA) translation and induce RNA degradation. Dicer is a ribonuclease needed for miRNA generation, and Dicer gene ablation first established their importance for lymphopoiesis²¹. Ectopic expression of miR-181 increased lymphocyte numbers, while the opposite resulted from miR-150 over-expression. Reciprocally, loss of miR-150 resulted in selective expansion of B-1 cells²². These findings showed that miR-150 may function as a negative regulator of B lymphopoiesis and play a role in B1 and B2 fate decisions.

MiR-125b is mostly expressed in HSCs; overexpression caused lymphoid biased HSCs and increased peripheral lymphocytes²³. This suggests that miR-125b may contribute to HSC heterogeneity and early lymphoid priming. Kuchen and colleagues performed comprehensive miRNA-, mRNA and ChIP-sequencing on various hematopoietic cells²⁴. They found miRNAs that are exclusively expressed in B lymphocytes and showed that miRNA expression was epigenetically regulated. This study only focused on B lineage differentiation stages after proB cells. Information of miRNA abundance and regulation during early lymphopoiesis such as ELP and CLP stages could be very valuable.

Roles are also being found for miRNAs in T lineage differentiation. As just one example, deep miRNA- and ChIP-sequencing of cells from various T lymphopoiesis stages revealed dynamic regulation of miRNAs²⁵. Some miRNAs that were abundant in BM precursors were down-regulated up to three fold in thymic T cell progenitors while levels of other miRNAs were increased.

Replenishing the thymus

T lymphocytes are derived from HSCs, but no HSCs are present in the thymus, and HSCs do not home to that organ when transplanted to normal, un-irradiated recipients. It was long believed that the thymus needs constant replenishment by bone marrow progenitors to make T cells. Those observations drove intensive effort to identify and characterize those progenitors. However, two interesting studies showed that DN stage progenitors resident in the thymus can sustain T lymphopoiesis for months, provided they face no competition from similar cells that are competent to respond to the cytokine IL-7^{26,27}. The experimental designs involved transplantation of wild-type thymuses to immunodeficient Rag2^{-/-}γc^{-/-}Kit^{w/w}, Rag2^{-/-}γc^{-/-} or IL-7Rα^{-/-} recipients with the surprising result that newly formed T cells were exported for some time. When the recipient mice were Kit^{w/w}, Rag2^{-/-}, the thymocytes were all replaced by host cells as expected. Competition among early progenitors had previously been appreciated by Petrie and colleagues²⁸.

The already difficult task of defining which marrow progenitors are most important for replenishing the thymus was made harder by these important results. However, much can be learned by comparison of the most primitive thymocytes to possible thymus seeding cells within bone marrow. That was achieved by Jacobsen and colleagues²⁹. Elegant single cell experiments revealed that early thymic progenitors resembled marrow equivalents with respect to gene expression patterns and differentiation potential. In both cases, loss of megakaryocyte-erythrocyte production occurred before myeloid, T and B lineage repopulating functions. This adds support to the “myeloid” based model long championed by Kawamoto and colleagues³⁰. In addition, Bhandoola and colleagues used a variety of

approaches to conclude that early thymocyte progenitors are a major source of granulocytes in the thymus³¹.

New information concerning NK cell generation

Like other lymphocytes, NK cell formation is marked by sequential acquisition of mature cell markers³². NK progenitors in bone marrow were previously defined as CD122 (IL-2R β)⁺NK1.1⁻CD49b⁻, but more primitive ones were described in two recent reports. Since the ID2 transcription factor is essential for NK production, Belz and colleagues exploited an ID2-GFP reporter to identify pre-pro NK cells with NK, but not B and T differentiation potential³³. They lacked Flt3, had distinctly high densities IL-7R α and, with the exception of CD244 and NKG2D, lacked mature NK cell markers. Two subsets of pre-proNK cells were distinguished by levels of c-Kit, but functionally similar. Given their high expression of IL-7R α and lineage restriction, it is likely that pre-pro NK cells represent relatively late stage CLP. An overlapping population of NK progenitors was identified with a different strategy³⁴. It is interesting that these marrow derived cells could produce NK cells on transplantation, but not when injected into the thymus. This supports the idea that NK cells are also formed in other sites and possibly via multiple differentiation pathways³⁵. In addition to ID2, Bcl11a deletion affects NK development as well as T and B cells. Interestingly, Bcl11a is expressed only in pre-pro NKa and CD122⁺NKP but not NKb progenitors, suggesting it might distinguish two different NK subsets²⁰.

Age-related changes and mechanisms

Diminished lymphopoietic potential of HSC occurs as a function of age. As covered elsewhere in this volume and our previous review, it is accompanied by altered ratios between HSC subsets¹ Figure 1 and (Eaves, this volume). Recent studies of the underlying mechanisms suggest that there are likely to be environmental as well as HSC-intrinsic changes.

High-resolution methods are now available for locating stem/progenitor cells within bone marrow sections. Exploitation of that approach revealed that aged HSC localize more distantly from endosteal areas than young HSC³⁶. As another possible example of environment and competition for space within lymphopoietic niches, depletion of long-lived mature B lymphocytes induced expansion of HSCs as well as CLP in the aged bone marrow³⁷.

Despite many years of investigation, it remains unclear how telomere dysfunction relates to ageing in the lympho-hematopoietic system. Suda and colleagues studied ataxia-telangiectasia mutated mice where premature ageing is induced through accumulation of reactive oxygen species. Telomerase activity protected HSC from age-related phenotypes, but independent of telomere length³⁸. While telomere shortening was reported to induce functional exhaustion in HSC in a cell-autonomous manner³⁹, telomere dysfunction in systemic environments may be more relevant to the age-related impairment of lymphopoiesis⁴⁰. Also, the basic leucine zipper transcription factor, ATF-like (BATF) was found to play an intermediary role for telomere attrition and DNA damage in HSC⁴¹. Once telomere dysfunction or DNA damage occurred in HSC, the induced BATF limited self-renewing proliferation. It also skewed their differentiation into CD150^{Lo} lymphoid-biased HSC. Interestingly, BATF transcripts and protein are increased in aged-HSC whose potential is highly myeloid-biased. This discrepancy may suggest that, while BATF protects aged HSC from myelogenous dysplasia, ageing affects more profoundly the lineage potential of HSC independent of telomere attrition.

RhoGTPase Cdc42 regulates cell polarity by modifying actin and tubulin organization. Geiger's group found that increased activity of Cdc42 in aged HSC paralleled loss of cell polarity⁴². Mice lacking RhoGAP protein, a highly selective inhibitor of Cdc activity, exhibited premature hematopoietic ageing phenotype, i. e. an increase of myeloid cells and a decrease of T and B lymphocytes. Importantly, treatment of aged HSC with a selective Cdc42 inhibitor restored not only HSC polarity but also their lymphopoietic potential⁴².

As methods for monitoring epigenetic regulation become more routinely used, evidence is accumulating to explain age-related changes⁴³. In that regard, it is noteworthy that pharmacological manipulation of Cdc42 activity reverted levels and patterns of histone H4 acetylation of aged HSC to those of young HSC⁴². It is interesting to consider that specific histone methylation and/or acetylation events might someday represent therapeutic targets.

Altered patterns during infections

HSC and progenitors express pathogen associated receptors (PPRs) such as Toll-like, RIG I-like, Nod-like and purinergic receptors^{44,45}. Although those receptors serve to sense components of bacteria, virus, and fungi, they may also detect endogenous ligands to elicit innate immunity. Accumulating evidence suggests these molecules can induce extramedullary hematopoiesis, migration of lymphoid progenitors and depletion of lymphopoiesis within marrow. Other features include myeloid skewing of hematopoiesis as well as exhaustion, cell cycle progression and deficient homing of HSC¹. While hematopoietic cells can directly respond to pathogen products, their properties are also influenced by inflammatory cytokines such as TNF α , IFN α , IFN γ and IL-6 but not IL-1 β ^{1,46}. The relative importance of direct versus indirect recognition of such substances is likely to depend on pathogens and routes of administration^{46,47}.

The Aryl hydrocarbon receptor (AHR) is a member of the basic helix loop helix family of transcription factors that merits further study in connection with hematopoiesis. A screen revealed that a small molecule promoted human HSC survival and xenotransplantation by antagonizing AHR⁴⁸. In contrast, the AHR ligand, TCDD increased numbers and proliferation of HSC, while compromising transplantation efficiency⁴⁹.

Purinergic receptors that sense extracellular nucleotides such as ATP, UTP and adenosine can evoke systemic immune responses. Interestingly, administration of ATP induces HSC proliferation and diminishes their long-term repopulation potential⁵⁰. These responses depended on Ca²⁺ signaling and were blocked by treatment in culture with myelopoietic cytokines. This is certain to be a fertile area of exploration, with implications for immune defense, cancer and regeneration of lymphopoiesis following myeloablation or transplantation.

Initiation of lymphopoiesis in humans

Studies of lymphopoiesis in humans, as with mice, have been unnecessarily complicated by nomenclature problems. For example, cells with distinctly different characteristics are referred to by the same name, and flow cytometry definitions evolve with time. Also, proprietary names are given to progenitors previously described by others. Added to this difficulty is the problem of efficiently observing human lymphocyte formation with culture or transplantation approaches. Nonetheless, significant progress is now being made in identifying human lymphoid progenitors (Figure 3).

IL-7 is essential for B lymphopoiesis in mice. Multiple studies agree that secondary up-regulation of Ebf-1 in response to IL-7 is essential for progression in the B lymphocyte lineage⁵¹. Further refinement showed that a relatively mature, Ly6D⁺subset of CLP is

particularly IL-7 dependent⁵¹. Unlike the situation in mice, IL-7 is neither required nor sufficient for human B lymphopoiesis. Furthermore, the IL-7R cannot be used as a marker for human B cell progenitors, and other milestones must be identified⁵².

Among progenitors isolated from human cord blood, CD7 expressing CD34⁺ cells exhibit the most T lymphocyte potential. CD10 is present on ones potent at generating B lymphocytes and is helpful for identifying the human equivalents of CLPs⁵². However, we found that cord blood and bone marrow progenitor cells express a wide range of CD10 densities. Increased levels correspond to more B lineage commitment and loss of ability to expand in culture⁵³. Another study reported that CD34⁺CD45RA⁺CD10⁻ multipotent progenitor cells from human cord blood could generate B cells without going through the CD10⁺CLP stage, suggesting multiple pathways of human B lymphopoiesis⁵⁴.

Dick and colleagues developed improved single cell culture assays for human progenitors⁵⁵. Using this tool, they identified Thy1⁻CD45RA⁺ multilineage progenitors (MLP) among the CD34⁺CD38⁻Lin⁻ fractions in cord blood and bone marrow. Similar to ELP/LMPP first discovered in mice^{56,57}, MLP gave rise to B, T, NK and dendritic cells as well as macrophages but lacked granulocyte and erythroid potential. They also used a xenotransplantation model to evaluate multilineage potential. Cord blood derived MLP generated CD19⁺B cells, CD33⁺granulocytes, CD14⁺monocytes and CD15⁺granulocytes but not T cells. Interestingly, some patients with monocytopenia lack phenotypic MLP as well as B, NK and dendritic cells, but do have T lymphocytes⁵⁸.

Others studied segregation of intermediate lymphoid progenitors in transition from CD34⁺CD38⁻ to CD10⁺CD38⁺CD34⁺ stages⁵⁹. A CD62L⁺ subset of the CD34⁺Lin⁻ fraction had B, T NK, monocyte and dendritic cell differentiation potential. Distinct from MLP, the CD10⁻CD62L⁺ population contains bi-potent B-NK progenitors and shows enhanced T cell and NK production. Moreover, compared to the CD10⁺ population, the CD62L⁺ progenitors have lower expression of B cell-related genes. Interestingly CD62L⁺ cells gave rise to CD10⁺ cells before CD19 expression in culture. This hierarchical expression of surface markers suggests that CD62L intermediate progenitors are upstream of CD10⁺MLP. Human lymphopoiesis may be marked by asynchronous patterns of gene expression as is the case for mice¹, but solid progress is being made in identifying those events.

Conclusion

Much has happened in the short interval since our last review¹, and this update still fails to mention some important findings. Even so, it is possible to see that microRNAs and epigenetic regulation will gain increased attention in connection with lymphopoiesis. Age-related declines in the process are now being effectively probed and may eventually be reversed. We already know that cytokine requirements for human and murine lymphocyte formation are different, as is the case for patterns of cell surface protein expression. Nonetheless, principals learned from experimental animals form a sound basis for dissecting the process in humans. One overall goal of this work is to have sufficient information to boost lymphocyte replacement during rebound from chemotherapy and transplantation. Another is to appreciate how imbalances occur during malignant and autoimmune diseases.

Acknowledgments

This work was supported by National Institutes of Health Grants AI020069 and HL107138 (P.W.K.). P.W.K. holds the William H. and Rita Bell Endowed Chair in Biomedical Research and is Scientific Director, Oklahoma Center for Adult Stem Cell Research. The authors thank Shelli Wasson for editorial assistance.

References

1. Zhang Q, Iida R, Shimazu T, Kincade PW. Replenishing B lymphocytes in health and disease. *Curr Opin Immunol.* 2012; 24:196–203. [PubMed: 22236696]
2. Welinder E, Ahsberg J, Sigvardsson M. B-lymphocyte commitment: identifying the point of no return. *Semin Immunol.* 2011; 23:335–340. [PubMed: 21944938]
3. Rothenberg EV. Transcriptional drivers of the T-cell lineage program. *Curr Opin Immunol.* 2012; 24:132–138. [PubMed: 22264928]
4. Copley MR, Beer PA, Eaves CJ. Hematopoietic stem cell heterogeneity takes center stage. *Cell Stem Cell.* 2012; 10:690–697. [PubMed: 22704509]
5. Ramírez J, Lukin K, Hagman J. From hematopoietic progenitors to B cells: mechanisms of lineage restriction and commitment. *Curr Opin Immunol.* 2010; 22:177–184. [PubMed: 20207529]
6. Ferreirós Vidal I, Carroll T, Taylor B, et al. Genome-wide identification of Ikaros targets elucidates its contribution to mouse B cell lineage specification and pre-B cell differentiation. *Blood.* 2013 in press. • Inducible expression systems and ChIP-seq analyses were used to identify a broad spectrum of genes associated with lymphopoiesis and a large proportion were directly bound by Ikaros family proteins.
7. Spooner CJ, Cheng JX, Pujadas E, Laslo P, et al. A recurrent network involving the transcription factors PU.1 and Gfi1 orchestrates innate and adaptive immune cell fates. *Immunity.* 2009; 31:576–586. [PubMed: 19818654]
8. Mansson R, Welinder E, Ahsberg J, et al. Positive intergenic feedback circuitry, involving EBF1 and FOXO1, orchestrates B-cell fate. *Proc Natl Acad Sci U S A.* 2012; 109:21028–21033. [PubMed: 23213261] • FOXO1 activates expression of EBF1 and then EBF1 reinforces FOXO1 expression in late CLP progenitors. Thus, they cooperate to fix B lineage identity.
9. Lin YC, Benner C, Mansson R, et al. Global changes in the nuclear positioning of genes and intra- and interdomain genomic interactions that orchestrate B cell fate. *Nat Immunol.* 2012; 13:1196–1204. [PubMed: 23064439] •• Previous knowledge of nuclear Ig gene localization has now been extended to include key regulators of B lymphopoiesis. A picture begins to emerge from this remarkable study of domains containing active and inactive genes.
10. Revilla-I-Domingo R, Bilic I, Vilagos B, et al. The B-cell identity factor Pax5 regulates distinct transcriptional programmes in early and late B lymphopoiesis. *EMBO J.* 2012; 31:3130–3146. [PubMed: 22669466] • Approximately one quarter of B lineage genes differentially expressed during late CLP to proB transition are Pax5 regulated. However, a remarkably different set of genes is controlled by Pax5 in mature B cells.
11. Zandi S, Ahsberg J, Tsapogas P, et al. Single-cell analysis of early B-lymphocyte development suggests independent regulation of lineage specification and commitment in vivo. *Proc Natl Acad Sci U S A.* 2012; 109:15871–15876. [PubMed: 23019372] • Ideas about the importance of Pax5 for initiating and maintaining B lineage stability have come from manipulation of cell lines and Bcl-2 transgenic mice. The conclusions are somewhat different from this new study, where *in vivo* and single cell assays were used in conjunction with a late CLP stage reporter. Of particular interest, B lineage specification and firm commitment were found to be distinct processes.
12. Simmons S, Knoll M, Drewell C, et al. Biphenotypic B-lymphoid/myeloid cells expressing low levels of Pax5: potential targets of BAL development. *Blood.* 2012; 120:3688–3698. [PubMed: 22927250] • Experimental reduction of Pax5 doses resulted in bi-phenotypic myeloid/lymphoid hybrid cells resembling those arising in lymphoid leukemia.
13. Zandi S, Mansson R, Tsapogas P, et al. EBF1 is essential for B-lineage priming and establishment of a transcription factor network in common lymphoid progenitors. *J Immunol.* 2008; 181:3364–3372. [PubMed: 18714008]
14. Lin YC, Jhunjhunwala S, Benner C, et al. A global network of transcription factors, involving E2A, EBF1 and Foxo1, that orchestrates B cell fate. *Nat Immunol.* 2010; 11:635–643. [PubMed: 20543837]
15. Vallespinós M, Fernández D, Rodríguez L, et al. B Lymphocyte commitment program is driven by the proto-oncogene c-Myc. *J Immunol.* 2011; 186:6726–6736. [PubMed: 21572027]
16. Fahl SP, Crittenden RB, Allman D, Bender TP. c-Myb is required for pro-B cell differentiation. *J Immunol.* 2009; 183:5582–5592. [PubMed: 19843942]

17. Greig KT, de Graaf CA, Murphy JM, et al. Critical roles for c-Myb in lymphoid priming and early B-cell development. *Blood*. 2010; 115:2796–2805. [PubMed: 20130238]
18. Seo W, Ikawa T, Kawamoto H, Taniuchi I. Runx1-Cbfb facilitates early B lymphocyte development by regulating expression of Ebf1. *J Exp Med*. 2012; 209:1255–1262. [PubMed: 22665574] • An essential early event in B lymphopoiesis, i.e. Ebf1 expression, is epigenetically regulated by Runx-1-Cbfb complexes.
19. Siggs OM, Arnold CN, Huber C, et al. The P4-type ATPase ATP11C is essential for B lymphopoiesis in adult bone marrow. *Nat Immunol*. 2011; 12:434–440. [PubMed: 21423172]
20. Yu Y, Wang J, Khaled W, et al. Bcl11a is essential for lymphoid development and negatively regulates p53. *J Exp Med*. 2012; 209:2467–2483. [PubMed: 23230003] • A checkpoint regulated by the Bcl11a transcription factor is essential for the survival of primitive cells that restore B, T and NK cell lineages.
21. Koralov SB, Muljo SA, Galler GR, et al. Dicer ablation affects antibody diversity and cell survival in the B lymphocyte lineage. *Cell*. 2008; 132:860–874. [PubMed: 18329371]
22. Xiao C, Calado DP, Galler G, et al. MiR-150 controls B cell differentiation by targeting the transcription factor c-Myb. *Cell*. 2007; 131:146–159. [PubMed: 17923094]
23. Ooi AG, Sahoo D, Adorno M, Wang Y, Weissman IL, Park CY. MicroRNA-125b expands hematopoietic stem cells and enriches for the lymphoid-balanced and lymphoid-biased subsets. *Proc Natl Acad Sci U S A*. 2010; 107:21505–21510. [PubMed: 21118986]
24. Kuchen S, Resch W, Yamane A, et al. Regulation of microRNA expression and abundance during lymphopoiesis. *Immunity*. 2010; 32:828–839. [PubMed: 20605486]
25. Kirigin FF, Lindstedt K, Sellars M, et al. Dynamic microRNA gene transcription and processing during T cell development. *J Immunol*. 2012; 188:3257–3267. [PubMed: 22379031] • Non-coding RNAs represent fertile ground for exploration of lymphopoietic regulation. As a first step, their expression patterns must be documented in stem/progenitor cells.
26. Martins VC, Ruggiero E, Schlenner SM, et al. Thymus-autonomous T cell development in the absence of progenitor import. *J Exp Med*. 2012; 209:1409–1417. [PubMed: 22778389] • Together with reference # 27, a long-held notion was demolished by these findings. The thymus indeed contains primitive progenitors that can extensively self-renew if not displaced by newly arriving ones from the bone marrow.
27. Peaudecerf L, Lemos S, Galgano A, et al. Thymocytes may persist and differentiate without any input from bone marrow progenitors. *J Exp Med*. 2012; 209:1401–1408. [PubMed: 22778388] • Together with reference # 26, a long-held notion was demolished by these findings. The thymus indeed contains primitive progenitors that can extensively self-renew if not displaced by newly arriving ones from the bone marrow.
28. Prockop SE, Petrie HT. Regulation of thymus size by competition for stromal niches among early T cell progenitors. *J Immunol*. 2004; 173:1604–1611. [PubMed: 15265888]
29. Luc S, Luis TC, Boukarabila H, et al. The earliest thymic T cell progenitors sustain B cell and myeloid lineage potential. *Nat Immunol*. 2012; 13:412–419. [PubMed: 22344248] •• If the thymus is replenished at some low rate from progenitors exported from bone marrow, there should be cells with similar properties in both sites. This exacting study shows such progenitors do exist and are unlikely to be fully lymphoid restricted.
30. Kawamoto H, Ikawa T, Masuda K, Wada H, Katsura Y. A map for lineage restriction of progenitors during hematopoiesis: the essence of the myeloid-based model. *Immunol Rev*. 2010; 238:23–36. [PubMed: 20969582]
31. De Obaldia ME, Bell JJ, Bhandoola A. Early T-cell progenitors are the major granulocyte precursors in the adult mouse thymus. *Blood*. 2013; 121:64–71. [PubMed: 23152541] •• Adding fuel to a contentious area, granulocytes are convincing made from early T progenitors present within the thymus.
32. Sun JC, Lanier LL. NK cell development, homeostasis and function: parallels with CD8⁺ T cells. *Nat Rev Immunol*. 2011; 11:645–657. [PubMed: 21869816]
33. Carotta S, Pang SH, Nutt SL, Belz GT. Identification of the earliest NK-cell precursor in the mouse BM. *Blood*. 2011; 117:5449–5452. [PubMed: 21422472]

34. Fathman JW, Bhattacharya D, Inlay MA, et al. Identification of the earliest natural killer cell committed progenitor in murine bone marrow. *Blood*. 2011; 118:5439–5447. [PubMed: 21931117]
35. Luther C, Warner K, Takei F. Unique progenitors in mouse lymph node develop into CD127+ NK cells: thymus-dependent and thymus-independent pathways. *Blood*. 2011; 117:4012–4021. [PubMed: 21355093]
36. Köhler A, Schmithorst V, Filippi MD, et al. Altered cellular dynamics and endosteal location of aged early hematopoietic progenitor cells revealed by time-lapse intravital imaging in long bones. *Blood*. 2009; 114(2):290–298. [PubMed: 19357397]
37. Keren Z, Naor S, Nussbaum S, et al. B-cell depletion reactivates B lymphopoiesis in the BM and rejuvenates the B lineage in aging. *Blood*. 2011; 117:3104–3112. [PubMed: 21228330]
38. Nitta E, Yamashita M, Hosokawa K, et al. Telomerase reverse transcriptase protects ATM-deficient hematopoietic stem cells from ROS-induced apoptosis through a telomere-independent mechanism. *Blood*. 2011; 117:4169–4180. [PubMed: 21297001]
39. Rossi DJ, Bryder D, Seita J, et al. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. *Nature*. 2007; 447:725–729. [PubMed: 17554309]
40. Song Z, Zhang J, Ju Z, Rudolph KL. Telomere dysfunctional environment induces loss of quiescence and inherent impairments of hematopoietic stem cell function. *Aging Cell*. 2012; 11:449–455. [PubMed: 22284665]
41. Wang J, Sun Q, Morita Y, et al. A differentiation checkpoint limits hematopoietic stem cell self-renewal in response to DNA damage. *Cell*. 2012; 148:1001–1014. [PubMed: 22385964]
42. Florian MC, Dörr K, Niebel A, et al. Cdc42 activity regulates hematopoietic stem cell aging and rejuvenation. *Cell Stem Cell*. 2012; 10:520–530. [PubMed: 22560076] • This interesting study describes an epigenetically regulated, age-related change in HSC that may be reversible with a drug.
43. Rando TA, Chang HY. Aging, rejuvenation, and epigenetic reprogramming: resetting the aging clock. *Cell*. 2012; 148:46–57. [PubMed: 22265401]
44. Nagai Y, Garrett KP, Ohta S, et al. Toll-like receptors on hematopoietic progenitor cells stimulate innate immune system replenishment. *Immunity*. 2006; 24(6):801–812. [PubMed: 16782035]
45. Rossi L, Salvestrini V, Ferrari D, Di Virgilio F, Lemoli RM. The sixth sense: hematopoietic stem cells detect danger through purinergic signaling. *Blood*. 2012; 120:2365–2375. [PubMed: 22786880]
46. Takizawa H, Boettcher S, Manz MG. Demand-adapted regulation of early hematopoiesis in infection and inflammation. *Blood*. 2012; 119:2991–3002. [PubMed: 22246037]
47. Boettcher S, Ziegler P, Schmid MA, et al. Cutting Edge: LPS-Induced Emergency Myelopoiesis Depends on TLR4-Expressing Nonhematopoietic Cells. *J Immunol*. 2012; 188:5824–5828. [PubMed: 22586037] • Complex interactions with infections agents likely involve direct and indirect recognition of pathogen products by hematopoietic stem/progenitor cells. This report nicely describes an example of the latter circumstance.
48. Boitano AE, Wang J, Romeo R, et al. Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science*. 2010; 329:1345–1348. [PubMed: 20688981]
49. Singh KP, Wyman A, Casado FL, Garrett RW, Gasiewicz TA. Treatment of mice with the Ah receptor agonist and human carcinogen dioxin results in altered numbers and function of hematopoietic stem cells. *Carcinogenesis*. 2009; 30:11–19. [PubMed: 18820284]
50. Barbosa CM, Leon CM, Nogueira-Pedro A, et al. Differentiation of hematopoietic stem cell and myeloid populations by ATP is modulated by cytokines. *Cell Death Dis*. 2011; 2:e165. [PubMed: 21633388]
51. Tsapogas P, Zandi S, Ahsberg J, et al. Il-7 mediates Ebf-1-dependent lineage restriction in early lymphoid progenitors. *Blood*. 2011; 118:1283–1290. [PubMed: 21652681]
52. Doulatov S, Notta F, Laurenti E, Dick JE. Hematopoiesis: a human perspective. *Cell Stem Cell*. 2012; 10:120–136. [PubMed: 22305562]
53. Ichii M, Oritani K, Yokota T, et al. The density of CD10 corresponds to commitment and progression in the human B lymphoid lineage. *PLoS One*. 2010; 5:e12954. [PubMed: 20886092]

54. Sanz E, Muñoz-A N, Monserrat J, et al. Ordering human CD34+CD10-CD19+ pre/pro-B-cell and CD19- common lymphoid progenitor stages in two pro-B-cell development pathways. *Proc Natl Acad Sci U S A*. 2010; 107:5925–5930. [PubMed: 20231472]
55. Doulatov S, Notta F, Eppert K, Nguyen LT, Ohashi PS, Dick JE. Revised map of the human progenitor hierarchy shows the origin of macrophages and dendritic cells in early lymphoid development. *Nat Immunol*. 2010; 11:585–593. [PubMed: 20543838]
56. Igarashi H, Gregory SC, Yokota T, Sakaguchi N, Kincade PW. Transcription from the RAG1 locus marks the earliest lymphocyte progenitors in bone marrow. *Immunity*. 2002; 17:117–130. [PubMed: 12196284]
57. Adolfsson J, Borge OJ, Bryder D, et al. Upregulation of Flt3 expression within the bone marrow Lin(–)Sca1(+)c-kit(+) stem cell compartment is accompanied by loss of self-renewal capacity. *Immunity*. 2001; 15:659–669. [PubMed: 11672547]
58. Bigley V, Haniffa M, Doulatov S, et al. The human syndrome of dendritic cell, monocyte, B and NK lymphoid deficiency. *J Exp Med*. 2011; 208:227–234. [PubMed: 21242295]
59. Kohn LA, Hao QL, Sasidharan R, et al. Lymphoid priming in human bone marrow begins before expression of CD10 with upregulation of L-selectin. *Nat Immunol*. 2012; 13:963–971. [PubMed: 22941246] • The pace of discovery relative to human B lymphopoiesis is finally accelerating. Consider this report together with recent findings from Dick and colleagues (see Figure 3 for more details).

Figure 1. Heterogeneity of hematopoietic stem cells

Subsets of hematopoietic stem cells (HSC) differ in terms of surface marker expression, dye efflux and function. In murine bone marrow, most are defined as $\text{Lin}^- \text{c-Kit}^+ \text{Sca-1}^+ \text{Flt3}^- \text{CD34}^- \text{CD150}^+ \text{CD48}^-$. Recent studies suggest there are at least three major HSC classes, distinguished according to the spectrum of blood cells they produced. That is, some are relatively balanced, others are biased to produce myeloid cells and still others preferentially generate lymphocytes. Ones with high densities of CD150 tend to be robust and myeloid biased. Additional variability relates to cell cycle status and potential for marrow engraftment on transplantation, as well as the kinetics and duration of blood cell formation. Stem cells can change with respect to composition and properties as a result of normal aging and during chronic infectious disease. Lymphoid progenitors arising from these HSC are themselves heterogeneous and acquire B cell properties in an asynchronous manner (Reproduced with permission from¹)

Figure 2. Micro RNAs in lymphocyte differentiation

Accumulating evidence indicates that miRNAs are actively involved in the regulation of lymphocyte differentiation^{21–24}. Kuchen et al identified a total of 49 miRNAs that are upregulated in lymphocytes or their progenitors²⁴. This figure highlights three miRNAs (MiR-181, MiR-150, and MiR125b) whose functions are indispensable for normal lymphocyte development. Arrows indicate positive regulation whereas blunt ended lines suggest that the function is normally inhibitory.

Figure 3. A comparison of B lymphopoiesis in humans and mice

While milestones of murine lymphopoiesis have been identified with many markers, it has been difficult to obtain comparable information for humans⁵². As one major complication, none of the cell surface proteins are expressed in conserved fashion. For example, CD10 instead of IL-7Rα is useful for defining human CLPs. A recent study showed that human CD62L⁺CD10⁺ progenitors have myeloid and lymphoid, but not erythroid or megakaryocyte potential⁵⁹. They can give rise to CD10⁺CLPs in culture, suggesting they are similar to mouse LMPP/ELP. CD10 density increases through human B lymphopoiesis and CD10^{Hi}CLPs may be equivalent to Ly6D⁺CLPs in mice⁵³. Patterns of CD45RA and CD10 expression help to delineate a relatively primitive form of CLP⁵⁵. Similar to the case in mice, human progenitors do not acquire markers in completely synchronous fashion and differentiate in a simple linear manner. However, complete loss of non-lymphoid potential appears to be a very late event in humans as compared to mice.