

Some hematopoietic stem cells are more equal than others

Hanno Hock

Hematopoietic stem cells (HSCs) save lives in routine clinical practice every day, as they are the key element in transplantation-based therapies for hematologic malignancies. The success of clinical stem cell transplantation critically relies on the ability of stem cells to reconstitute the hematopoietic system for many decades after the administration of the powerful chemotherapy and/or irradiation that is required to eradicate malignant cells, but also irreversibly ablates patients' own blood forming capacity. Surprisingly, despite enormous efforts and continuous progress in the field, our understanding of the basic biology of HSCs is still rather incomplete. Several recent studies substantially refine our understanding of the cells at the very top of the hematopoietic hierarchy, and suggest that we may need to revise the criteria we typically use to identify and define HSCs.

In 1996, a group led by Hiromitsu Nakauchi in Tokyo was the first to develop a method that permitted sufficient enrichment of murine HSCs to allow individual stem cells to be transplanted at high frequency and to formally demonstrate that single cells harbor the potential to replicate themselves (self-renewal) and give rise to the entire repertoire of differentiated blood cell types (multilineage potential) for long periods of time (Osawa et al., 1996). Since then, studies from several groups investigating hematopoiesis using single-cell transplantation established that individual HSCs display heterogeneous stable “characters,” in regard to the balance of lineages they produce, their self-renewal capacity, and their engraftment kinetics (Fig. 1; Muller-Sieburg et al., 2002, 2004; Ema et al., 2005; Dykstra et al., 2006). In this issue, another group led by Nakauchi (Morita et al., 2010) set out to prospectively isolate subtypes of HSCs.

The team initially screened >100 surface markers to identify candidates showing differential expression on already highly purified HSCs (CD34⁺c-Kit⁺Sca1⁺Lineage marker[−]). They

subsequently tested those 15 markers that did show differential expression in transplantation assays. CD150 (also known as signaling lymphocyte activation family member 1 [SlamF1]), which was initially identified as a stem cell marker by Kiel et al. (2005), emerged as perhaps the most interesting candidate (Morita et al., 2010). Single-cell transplantation of HSCs distinguished by their level of CD150 surface expression yielded important insights that will be discussed in the context of other recently published findings.

Abundant CD150 predicts robust HSC self-renewal

Bone marrow cells from all 13 mice transplanted with single CD150^{high} HSCs gave rise to hematopoiesis after secondary transplantation (Morita et al., 2010). In contrast, only a minority of single CD150^{med} or CD150^{neg} HSCs gave rise to significant hematopoiesis in secondary recipients (Morita et al., 2010). These findings dovetail with data demonstrating (with an entirely different HSC enrichment strategy) that CD150 expression predicts successful repopulation of secondary recipients (Kent et al., 2009). Together, these data put earlier controversies (Akala et al., 2008; Kiel et al., 2008; Weksberg et al., 2008) about whether CD150^{neg} HSCs exist into perspective and suggest that the answer is to some

degree arbitrary. A proportion of HSCs lacking CD150 meet the conventional criteria for HSC activity in primary recipients, but these cells largely lack durable self-renewal in secondary transplant assays. High expression of CD150 marks HSCs with the most potent self-renewal capacity.

CD150 levels predict myeloid versus lymphoid reconstitution potential

Morita et al. (2010) further demonstrate that single CD150^{high} HSCs display robust myeloid reconstitution potential upon transplantation, whereas CD150^{neg} HSCs mediate only faint myeloid, but superior lymphoid, reconstitution; these patterns appeared stable in secondary transplantations. Recently, three other groups independently reported similar findings, even though the enrichment strategies for HSCs were quite dissimilar except for the use of CD150 (Kent et al., 2009; Beerman et al., 2010; Challen et al., 2010). Specifically, Kent et al. (2009) examined lymphoid versus myeloid reconstitution patterns associated with the absence or presence of CD150 on HSCs (defined as CD45⁺EPCR⁺CD48[−]; Kiel et al., 2005; Balazs et al., 2006) and report a strong predominance of myeloid or lymphoid reconstitution after transplantation of CD150⁺ or CD150[−] cells, respectively. Challen et al. (2010) demonstrated that myeloid and lymphoid-biased HSCs can be purified based on Hoechst dye efflux in combination with the absence of lineage markers and the presence of Sca-1 and c-Kit; further separation of these HSCs based on CD150 expression enhanced discrimination of the lineage bias. Finally, Beerman et al. (2010) used an HSC isolation strategy similar to that used by

H.H. is at the Cancer Center and Center for Regenerative Medicine, Massachusetts General Hospital, Harvard Medical School, and Harvard Stem Cell Institute, Boston, MA 02114.

CORRESPONDENCE

H.H.: Hock.Hanno@mgh.harvard.edu

© 2010 Hock This article is distributed under the terms of an Attribution-Noncommercial-Share Alike-No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms>). After six months it is available under a Creative Commons License (Attribution-Noncommercial-Share Alike 3.0 Unported license, as described at <http://creativecommons.org/licenses/by-nc-sa/3.0/>).

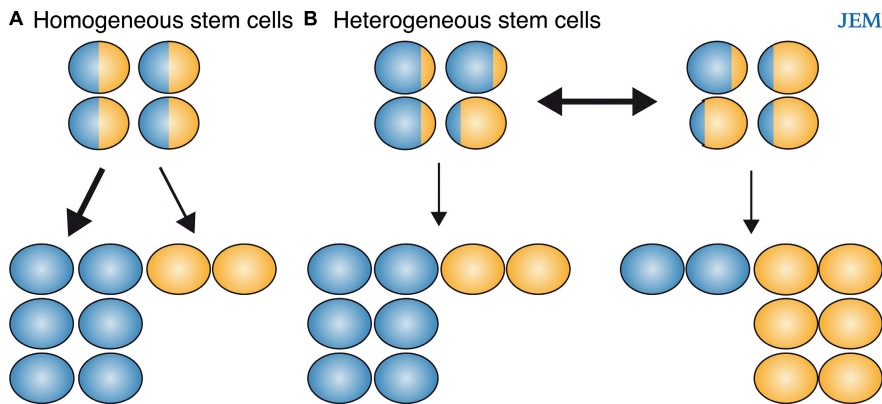


Figure 1. Homogeneous versus heterogeneous HSC populations. (A) Traditional scheme of a uniform HSC population in which each cell has a similar propensity to give rise to different lineage-committed progeny (top). Propensities to generate different lineages are symbolized by different colors. The composition of pool of differentiated cells (bottom) is determined by different production rates beyond the stem cell level (indicated by thickness of arrows). (B) New findings suggest that the propensities of different HSCs to give rise to different lineages are fixed, so the lineage mix in the periphery may be influenced by the composition of the HSC pool. Morita et al. (2010) show that different lineage-biased HSCs can be enriched using the surface marker CD150.

Nakauchi's group and detected the same lineage bias associated with CD150 expression. Interestingly, this study revealed that the proportion of CD150^{high} HSCs strikingly increases with aging, whereas the proportion of CD150^{neg} or CD150^{low} HSCs strikingly diminishes with aging, suggesting that the well documented aging-related compromise of lymphopoiesis (Sudo et al., 2000; Rossi et al., 2005) is not caused by loss of lymphoid potential by a homogeneous population of stem cells, but rather by expansion of HSC populations with predominantly myeloid potential (Beerman et al., 2010). This model is also supported by recent experiments using limiting dilution transplantation of total bone marrow (Cho et al., 2008). Collectively, these data suggest that the output of different blood cell types by the hematopoietic system may be influenced by a balance of coexisting stem cell populations with different lineage propensities. The proportions of different stem cells appear to impact the final composition of differentiated blood cell populations even before the separation of distinct lineages occurs (Fig. 1).

CD150^{high} HSCs reside at the top of the HSC hierarchy

If different HSC populations coexist, an important question is whether they exist in a hierarchy. In principle, the

different populations might be able to convert into each other, or they may derive from a parental stem cell that may or may not persist into adulthood (Challen et al., 2010). Both Morita et al. (2010) and Beerman et al. (2010) demonstrate that CD150^{high} HSCs can give rise to CD150^{high} HSCs (themselves), as well as to CD150^{low} and CD150^{neg} HSCs after transplantation, but that CD150^{low} and CD150^{neg} HSCs fail to give rise to CD150^{high} HSCs. Thus, CD150^{high} HSCs represent the top of the hierarchy. Yet, it is unclear at this point whether CD150^{high} HSCs continuously replenish CD150^{low} and CD150^{neg} HSCs during the steady state in adult bone marrow. Beerman et al. (2010) show that CD150^{low} HSCs cycle very infrequently. Consistent with this, using histone 2B-GFP label retention Foudi et al. (2009) demonstrated that CD150^{neg} HSCs (Lineage marker⁻c-Kit⁺Sca-1⁺CD48⁻) turn over almost as slowly as CD150⁺ HSCs. Hence, it is possible that this population can be maintained for long periods of time, even with limited self-renewal capacity. During normal aging, CD150^{high} HSC populations expand while CD150^{low} HSC populations diminish, suggesting that they are differentially regulated (Beerman et al., 2010). More directly, this notion is supported by the demonstration that

lineage-biased HSC subtypes respond differently to transforming growth factor- β 1 (Challen et al., 2010). Deciphering the regulation of different HSC populations and investigating their potential cross talk is an important task for the future.

CD150^{neg} HSCs: a step toward lymphoid-primed multipotent progenitors

CD150^{neg} HSCs harbor an impressive capacity to reconstitute the lymphoid system (Kent et al., 2009; Beerman et al., 2010; Challen et al., 2010; Morita et al., 2010). This may be interpreted as a first step toward lymphoid differentiation. However, their partially preserved self-renewal and the absence of expression of Flt-3 clearly distinguish CD150^{neg} HSCs from the first known major stage of lymphoid differentiation, the lymphoid-primed multipotent progenitor (LMPP; Adolfsson et al., 2005). Supporting a close developmental relationship between CD150^{neg} HSCs and LMPPs, CD150^{low} HSCs give rise to LMPPs after transplantation much more efficiently than CD150^{high} HSCs (Beerman et al., 2010). In addition, CD150^{neg} HSCs harbor diminished erythroid-megakaryocytic potential in vitro (Morita et al., 2010). This is intriguing because loss of erythroid-megakaryocytic potential, even if controversial (Forsberg et al., 2006), was described as a hallmark trait of LMPPs (Adolfsson et al., 2005; Luc et al., 2007). Thus, it appears that self-renewal is not always entirely extinguished during the initial steps toward lineage differentiation.

"Latent" CD150^{high} HSCs

The analysis of reconstitution patterns performed by Morita et al. (2010) suggests that the CD150^{high} HSC population is still heterogeneous. In the future, new markers may resolve this population even further. They report two patterns of engraftment of CD150^{high} HSCs that challenge conventional expectations. First, some stem cells appear to exclusively give rise to myeloid cells and, nevertheless, are capable of repopulating secondary hosts. Second, up to

10% of CD150^{high} HSCs represent “latent” stem cells, which did not give rise to detectable progeny for at least 3 mo after transplantation, and then produced only low levels of myeloid reconstitution in the primary host. Yet, they gave rise to robust, progressive multilineage engraftment after secondary transplantation. The authors speculate that “latent” HSCs may overlap with recently described highly quiescent label-retaining HSCs (Wilson et al., 2008; Foudi et al., 2009). Such label-retaining HSCs, like “latent” HSCs, exhibited much stronger reconstitution activity after secondary transplantation (Wilson et al., 2008; Foudi et al., 2009). Single-cell transplantation of label-retaining HSCs will be necessary to definitively clarify their overlap with “latent” HSCs. Whether or not they are quiescent, the existence of the latent reconstitution patterns demonstrates that not all HSCs contribute to blood formation at all times. This observation is reminiscent of retroviral marking experiments, which suggested that hematopoiesis at any given time is sustained by only very few clones (Lemischka et al., 1986; Jordan and Lemischka, 1990). These experiments support a clonal succession model, in which most of the cells within the HSC pool remain quiescent, periodically releasing active clones to sustain blood production, as previously active clones exhaust their proliferative capacity. However, a clonal succession model was difficult to reconcile with data from chimeric mice made with different embryonic stem cell founders (Harrison et al., 1987) that showed that hematopoiesis was sustained simultaneously by large numbers of HSCs derived from different founder cells. Nevertheless, the latter observations do not prove that all or even the majority of HSCs produce blood at all times. It is tempting to speculate that latent stem cell behavior may be even more common in the nontransplanted host in the absence of stimulation from cytopenias resulting from myeloablative irradiation. It will likely be possible in the future to directly investigate such issues using conditional clonal genetic marking strategies that do not involve transplantation.

Limitations of current definitions for HSCs

At least in primary transplant recipients, “latent” HSCs do not meet widely accepted operational criteria for HSC activity, which set the bar at a >1% contribution to myeloid and lymphoid progeny in the peripheral blood for 16 wk (Morrison and Weissman, 1994; Miller and Eaves, 1997; Ema et al., 2005). As such cells are undoubtedly very potent HSCs, Morita et al. (2010) reasonably emphasize that current criteria for defining HSCs may need to be reexamined. In fact, defining HSC activity based on peripheral blood analysis alone is also problematic for biological reasons. Although granulocytes are short-lived so that their presence in the blood, indeed, indicates ongoing active cell production, the situation is much more complicated with blood B and T lymphocytes. Conditional ablation of B and T cell production by time-controlled disruption of the *recombination-activating gene-2* gene has shown that peripheral B and T lymphocytes are maintained for very long time periods, even in the absence of any new production (Hao and Rajewsky, 2001; Bourgeois et al., 2008). For example, naive follicular B cells are only gradually lost, with a half-life of ~4.5 mo, and some smaller B cell subsets remain stable indefinitely (Hao and Rajewsky, 2001). As a result, using 1% lymphoid reconstitution as a criterion may lead to considerable overestimates of HSC properties, particularly for HSC populations with predominantly lymphoid output. Thus, there is reason to critically rethink current definitions of HSCs.

Conclusion

It is emerging that heterogeneous HSC populations in the bone marrow coexist and coordinately give rise to hematopoiesis. In combination with other markers, the SLAM family member CD150 allows for the prospective enrichment of HSC populations with distinct characteristics. High expression of CD150 appears to identify cells at the very top of the hematopoietic hierarchy, as it is correlated with high self-renewal and marks the only cells with

the potential to give rise to themselves, as well as to all other stem cell populations after transplantation. Absence or low expression of CD150 on HSCs is associated with the acquisition of powerful lymphoid reconstitution potential. In the future, it will be important to further define the precise regulation, developmental relationships, and cross talk between different HSC populations, as well as to explore their role in disease.

This work was supported by National Cancer Institute grant R01CA122726.

REFERENCES

- Adolfsson, J., R. Månsson, N. Buza-Vidas, A. Hultquist, K. Liuba, C.T. Jensen, D. Bryder, L. Yang, O.J. Borge, L.A. Thoren, et al. 2005. Identification of Flt3⁺ lymphomyeloid stem cells lacking erythro-megakaryocytic potential: a revised road map for adult blood lineage commitment. *Cell*. 121:295–306. doi:10.1016/j.cell.2005.02.013
- Akala, O.O., I.K. Park, D. Qian, M. Pihajla, M.W. Becker, and M.F. Clarke. 2008. Long-term haematopoietic reconstitution by Trp53^{-/-}/p16Ink4a^{-/-}/p19Arf^{-/-} multipotent progenitors. *Nature*. 453:228–232. doi:10.1038/nature06869
- Balazs, A.B., A.J. Fabian, C.T. Esmon, and R.C. Mulligan. 2006. Endothelial protein C receptor (CD201) explicitly identifies hematopoietic stem cells in murine bone marrow. *Blood*. 107:2317–2321. doi:10.1182/blood-2005-06-2249
- Beerman, I., D. Bhattacharya, S. Zandi, M. Sigvardsson, I.L. Weissman, D. Bryder, and D.J. Rossi. 2010. Functionally distinct hematopoietic stem cells modulate hematopoietic lineage potential during aging by a mechanism of clonal expansion. *Proc. Natl. Acad. Sci. USA*. 107:5465–5470. doi:10.1073/pnas.1000834107
- Bourgeois, C., Z. Hao, K. Rajewsky, A.J. Potocnik, and B. Stockinger. 2008. Ablation of thymic export causes accelerated decay of naive CD4 T cells in the periphery because of activation by environmental antigen. *Proc. Natl. Acad. Sci. USA*. 105:8691–8696. doi:10.1073/pnas.0803732105
- Challen, G.A., N.C. Boles, S.M. Chambers, and M.A. Goodell. 2010. Distinct hematopoietic stem cell subtypes are differentially regulated by TGF-beta1. *Cell Stem Cell*. 6:265–278. doi:10.1016/j.stem.2010.02.002
- Cho, R.H., H.B. Sieburg, and C.E. Muller-Sieburg. 2008. A new mechanism for the aging of hematopoietic stem cells: aging changes the clonal composition of the stem cell compartment but not individual stem cells. *Blood*. 111:5553–5561. doi:10.1182/blood-2007-11-123547
- Dykstra, B., J. Ramunas, D. Kent, L. McCaffrey, E. Szumsky, L. Kelly, K. Farn, A. Blaylock, C. Eaves, and E. Jarvis. 2006. High-resolution video monitoring of hematopoietic stem cells cultured in single-cell arrays

- identifies new features of self-renewal. *Proc. Natl. Acad. Sci. USA*. 103:8185–8190. doi:10.1073/pnas.0602548103
- Ema, H., K. Sudo, J. Seita, A. Matsubara, Y. Morita, M. Osawa, K. Takatsu, S. Takaki, and H. Nakauchi. 2005. Quantification of self-renewal capacity in single hematopoietic stem cells from normal and Lnk-deficient mice. *Dev. Cell*. 8:907–914. doi:10.1016/j.devcel.2005.03.019
- Forsberg, E.C., T. Serwold, S. Kogan, I.L. Weissman, and E. Passegué. 2006. New evidence supporting megakaryocyte-erythrocyte potential of flk2/flt3+ multipotent hematopoietic progenitors. *Cell*. 126:415–426. doi:10.1016/j.cell.2006.06.037
- Foudi, A., K. Hochedlinger, D. Van Buren, J.W. Schindler, R. Jaenisch, V. Carey, and H. Hock. 2009. Analysis of histone 2B-GFP retention reveals slowly cycling hematopoietic stem cells. *Nat. Biotechnol.* 27:84–90. doi:10.1038/nbt.1517
- Hao, Z., and K. Rajewsky. 2001. Homeostasis of peripheral B cells in the absence of B cell influx from the bone marrow. *J. Exp. Med.* 194:1151–1164. doi:10.1084/jem.194.8.1151
- Harrison, D.E., C. Lerner, P.C. Hoppe, G.A. Carlson, and D. Alling. 1987. Large numbers of primitive stem cells are active simultaneously in aggregated embryo chimeric mice. *Blood*. 69:773–777.
- Jordan, C.T., and I.R. Lemischka. 1990. Clonal and systemic analysis of long-term hematopoiesis in the mouse. *Genes Dev.* 4:220–232. doi:10.1101/gad.4.2.220
- Kent, D.G., M.R. Copley, C. Benz, S. Wöhrer, B.J. Dykstra, E. Ma, J. Cheyne, Y. Zhao, M.B. Bowie, Y. Zhao, et al. 2009. Prospective isolation and molecular characterization of hematopoietic stem cells with durable self-renewal potential. *Blood*. 113:6342–6350. doi:10.1182/blood-2008-12-192054
- Kiel, M.J., O.H. Yilmaz, T. Iwashita, O.H. Yilmaz, C. Terhorst, and S.J. Morrison. 2005. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell*. 121:1109–1121. doi:10.1016/j.cell.2005.05.026
- Kiel, M.J., O.H. Yilmaz, and S.J. Morrison. 2008. CD150+ cells are transiently reconstituting multipotent progenitors with little or no stem cell activity. *Blood*. 111:4413–4414, author reply:4414–4415. doi:10.1182/blood-2007-12-129601
- Lemischka, I.R., D.H. Raulet, and R.C. Mulligan. 1986. Developmental potential and dynamic behavior of hematopoietic stem cells. *Cell*. 45:917–927. doi:10.1016/0092-8674(86)90566-0
- Luc, S., N. Buza-Vidas, and S.E. Jacobsen. 2007. Biological and molecular evidence for existence of lymphoid-primed multipotent progenitors. *Ann. N. Y. Acad. Sci.* 1106:89–94. doi:10.1196/annals.1392.023
- Miller, C.L., and C.J. Eaves. 1997. Expansion in vitro of adult murine hematopoietic stem cells with transplantable lymphomyeloid reconstituting ability. *Proc. Natl. Acad. Sci. USA*. 94:13648–13653. doi:10.1073/pnas.94.25.13648
- Morita, Y., H. Ema, and H. Nakauchi. 2010. Heterogeneity and hierarchy within the most primitive hematopoietic stem cell compartment. *J. Exp. Med.* 207:1173–1182.
- Morrison, S.J., and I.L. Weissman. 1994. The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. *Immunity*. 1:661–673. doi:10.1016/1074-7613(94)90037-X
- Müller-Sieburg, C.E., R.H. Cho, M. Thoman, B. Adkins, and H.B. Sieburg. 2002. Deterministic regulation of hematopoietic stem cell self-renewal and differentiation. *Blood*. 100:1302–1309.
- Muller-Sieburg, C.E., R.H. Cho, L. Karlsson, J.F. Huang, and H.B. Sieburg. 2004. Myeloid-biased hematopoietic stem cells have extensive self-renewal capacity but generate diminished lymphoid progeny with impaired IL-7 responsiveness. *Blood*. 103:4111–4118. doi:10.1182/blood-2003-10-3448
- Osawa, M., K. Hanada, H. Hamada, and H. Nakauchi. 1996. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science*. 273:242–245. doi:10.1126/science.273.5272.242
- Rossi, D.J., D. Bryder, J.M. Zahn, H. Ahlenius, R. Sonu, A.J. Wagers, and I.L. Weissman. 2005. Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc. Natl. Acad. Sci. USA*. 102:9194–9199. doi:10.1073/pnas.0503280102
- Sudo, K., H. Ema, Y. Morita, and H. Nakauchi. 2000. Age-associated characteristics of murine hematopoietic stem cells. *J. Exp. Med.* 192:1273–1280. doi:10.1084/jem.192.9.1273
- Weksberg, D.C., S.M. Chambers, N.C. Boles, and M.A. Goodell. 2008. CD150+ side population cells represent a functionally distinct population of long-term hematopoietic stem cells. *Blood*. 111:2444–2451. doi:10.1182/blood-2007-09-115006
- Wilson, A., E. Laurenti, G. Oser, R.C. van der Wath, W. Blanco-Bose, M. Jaworski, S. Offner, C.F. Dunant, L. Eshkind, E. Bockamp, et al. 2008. Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. *Cell*. 135:1118–1129. doi:10.1016/j.cell.2008.10.048