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Experimental Hematology

Experimental Hematology 2014;42:74–82

Anniversary Review Series: Perspectives on the modern exploration of Experimental Hematology

Heterogeneity and hierarchy of hematopoietic stem cells

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(Received 3 July 2013; revised 16 October 2013; accepted 4 November 2013)

Hematopoietic stem cells (HSCs) are a more heterogeneous population than previously thought. Extensive analysis of reconstitution kinetics after transplantation allows a new classifications of HSCs based on lineage balance. Previously unrecognized classes of HSCs, such as myeloid- and lymphoid-biased HSCs, have emerged. However, varying nomenclature has been used to describe these cells, promoting confusion in the field. To establish a common nomenclature, we propose a reclassification of short-, intermediate-, and long-term (ST, IT, and LT) HSCs defined as: ST $<$ 6 months, IT $>$ 6 months, and LT $>$ 12. We observe that myeloid-biased HSCs or α cells overlap with LT-HSCs, whereas lymphoid-biased HSCs or γ/δ cells overlap with ST-HSCs, suggesting that HSC lifespan is linked to cell differentiation. We also suggest that HSC heterogeneity prompts reconsideration of long-term (>4 months) multilineage reconstitution as the gold standard for HSC detection. In this review, we discuss relationships among ST-, IT-, and LT-HSCs relevant to stem cell heterogeneity, hierarchical organization, and differentiation pathways. © **2014 ISEH - Society for Hematology and Stem Cells. Published by Elsevier Inc.**

Hematopoietic stem cells (HSCs) are defined as cells with self-renewal and differentiation potential [1]. Accumulated data show that HSCs are a heterogeneous population in multiple aspects, including their degree of self-renewal [2,3], differentiation manner [4,5], and lifespan [6–8]. Retroviral marking studies indicate that HSCs clonally give rise to all blood lineages and self-renew (a finding that represents definitive proof for the existence of HSCs in mouse bone marrow) [9–11]. Moreover, marking techniques have been used to demonstrate various patterns of reconstitution kinetics after HSC transplantation. Interestingly, some clones preferentially reconstitute a lymphoid lineage, whereas others preferentially reconstitute a myeloid one [12].

Lineage reconstitution kinetics have been examined extensively in mice transplanted with cultured bone marrow cells or with limiting doses of bone marrow cells freshly obtained from adult mice [13–15]. These studies suggest the presence of myeloid-biased HSCs (My-bi HSCs), lymphoid-biased HSCs (Ly-bi HSCs), and balanced HSCs (Bala HSCs). On the other hand, α, β, γ, and δ cells have been defined by others [16,17]. The presence of all these HSCs has been verified by single-cell transplantation [16,18–20]. Both types of classification are defined based on myeloid and lymphoid reconstitution ratios, but the criteria used to make these classifications differ fundamentally from one another (discussed later).

In this study, we propose a third classification, LT-, IT-, and ST-HSCs, based on reconstitution time periods [21]. We then examine the relationship of the three classification systems and discuss how different HSC classes are related to one another in the hematopoietic hierarchy. These

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Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.exphem.2013.11.004.

http://dx.doi.org/10.1016/j.exphem.2013.11.004

comparisons support that HSC lifespan is tightly associated with lineage contribution [13-17].

In the prevailing bifurcation model [22], following loss of self-renewal potential HSCs give rise to multipotent progenitors (MPPs), which commit to either myeloid or lymphoid lineages exclusively. According to this model, this is the first step in lineage commitment. However, MPPs or their progenitor equivalents have not been identified at the single-cell level. Other studies suggest that loss of lymphoid differentiation potential could occur as one of the first lineage commitment steps [23,24]. It is time to consider a more comprehensive differentiation model. We propose a new differentiation model consisting of LT-, IT-, and ST-HSCs.

HSC classifications

My-bi, Bala, and Ly-bi HSCs

Muller-Sieburg et al. [13,14] have defined My-bi, Bala, and Ly-bi HSCs based on the ratio of lymphoid to myeloid cells (the L/M ratio). The proportions of lymphoid to myeloid cells are calculated among test-donor-derived cells (Supplementary Figure E1, online only, available at www.exphem.org); thus, (% lymphoid cells) + (% myeloid cells) = 100. In this classification, long-term reconstitution is assessed 20 weeks or more after transplantation [13–15]. Transplanted cells are designated My-bi HSCs when the L/M ratio is less than 3 and Ly-bi HSCs when it exceeds 10. Cells are considered Bala HSCs when the L/M ratio exceeds 3 but is less than 10 [13–15].

These types of HSCs were detected basically using in vivo limiting dilution analysis [13–15]. Later, a different group reported that Ly-HSCs and My-HSCs are enriched in the upper and lower portions of SP, respectively, and successfully accomplished single-cell reconstitution with these HSCs [18]. Platelet-biased HSCs have also been reported as a My-bi subclass potentially residing at the apex of the hematopoietic hierarchy [25].

Figure 1 shows typical reconstitution patterns seen following transplantation of single My-bi, Bala, and Ly-bi HSCs. My-bi HSCs reconstitute the myeloid lineage after varying latencies, followed by gradual reconstitution of the lymphoid lineage (Fig. 1A). Thus, the myeloid lineage is more significantly reconstituted at early stages of reconstitution. In contrast, Ly-bi HSCs show both myeloid and lymphoid lineage reconstitution from early stages (Fig. 1C). Ly-bi HSCs reconstitute the myeloid lineage to a less extent than the lymphoid lineage. Myeloid reconstitution is often detectable for only a few months, but lymphoid reconstitution can persist relatively longer. Bala HSCs reconstitute the lymphoid lineage soon after the myeloid lineage (Fig. 1B). The proportions of myeloid and lymphoid lineage cells resemble those seen in the peripheral blood of normal mice [13–15]. Many investigators consider Bala HSCs to be typical HSCs, which might account for why the presence of My-bi HSCs and Ly-bi HSCs has been overlooked [4].

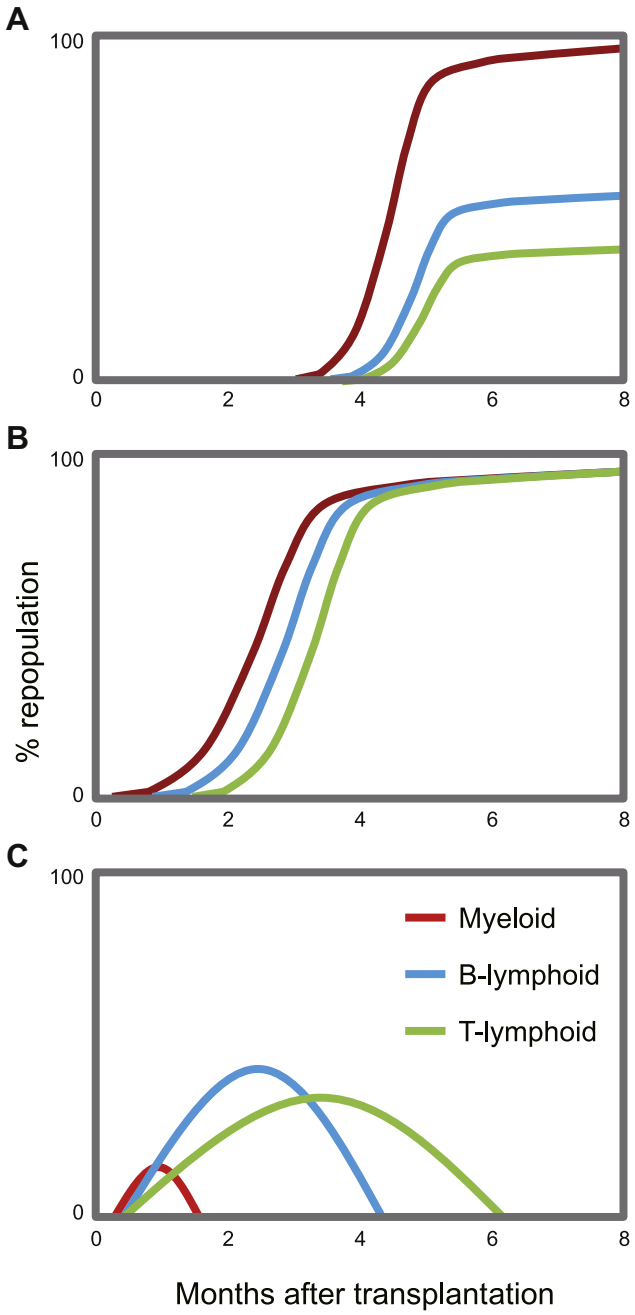

Figure 1. Reconstitution kinetics of My-bi, Bala, and Ly-bi HSCs. Shown are typical reconstitution patterns seen following single-cell transplantation of My-bi **(A)**, Bala **(B)**, and Ly-bi **(C)** HSCs, based on published data [19].

α, β, and γ/δ cells

Eaves et al. have defined α, β, γ, and δ cells as the percentage of myeloid chimerism relative to that of lymphoid chimerism (the M/L ratio) [16,17]. The M/L ratio is not simply the reciprocal of the L/M ratio described by