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MafB Restricts M-CSF-Dependent Myeloid Commitment Divisions of Hematopoietic Stem Cells

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SUMMARY

While hematopoietic stem cell (HSC) self-renewal is well studied, it remains unknown whether distinct control mechanisms enable HSC divisions that generate progeny cells with specific lineage bias. Here, we report that the monocytic transcription factor MafB specifically restricts the ability of M-CSF to instruct myeloid commitment divisions in HSCs. MafB deficiency specifically enhanced sensitivity to M-CSF and caused activation of the myeloid master-regulator PU.1 in HSCs in vivo. Single-cell analysis revealed that reduced MafB levels enabled M-CSF to instruct divisions producing asymmetric daughter pairs with one PU.1^{+} cell. As a consequence, *MafB*$^{-/-}$ HSCs showed a PU.1 and M-CSF receptor-dependent competitive repopulation advantage specifically in the myelomonocytic, but not T lymphoid or erythroid, compartment. Lineage-biased repopulation advantage was progressive, maintained long term, and serially transplantable. Together, this indicates that an integrated transcription factor/cytokine circuit can control the rate of specific HSC commitment divisions without compromising other lineages or self-renewal.

INTRODUCTION

The different short-lived cell types of mammalian blood are continuously regenerated from a small population of hematopoietic stem cells (HSCs) in the bone marrow (Bryder et al., 2006). Although a significant proportion of HSCs with long-term reconstitution potential is predominantly quiescent or divides infrequently (Wilson et al., 2008), HSCs need to enter the cycle to continuously regenerate mature blood cells in a correctly balanced ratio or to replenish the stem cell pool under stress conditions. Cell division of HSCs may thus result in self-renewal divisions or the production of more differentiated progeny (Orford and Scadden, 2008). Although such downstream progenitors still retain a high degree of multipotency, recent advances in their characterization also suggest that early diversification into cells with distinct lineage bias can occur at the most primitive stem and precursor cell level (Dykstra et al., 2007; Iwasaki and Akashi, 2007; Luc et al., 2008). However, the mechanisms controlling such specific lineage engagement divisions remain elusive.

Several cellular regulators have been identified that can either promote or restrict HSC cycling, but their mutation in genetic models exclusively affected self-renewal (Orford and Scadden, 2008; Zon, 2008). By contrast, regulators that selectively control lineage-specific commitment divisions of HSCs have not been identified. In this context, the importance of both transcription factor and cytokine signaling for lineage engagement has been invoked (Metcalf, 2007, 2008; Orkin and Zon, 2008; Sieweke and Graf, 1998; Zhang and Lodish, 2008), but no clear mechanism has emerged as to how these two critical control elements might be integrated. Transcription factors with effects on stem cell cycling so far were exclusively found to affect self-renewal divisions (Orford and Scadden, 2008; Zon, 2008). As for cytokine receptors, several of them are expressed on primitive hematopoietic stem and precursor cells (Akashi et al., 2003; Hu et al., 1997; Miyamoto et al., 2002), but it has been a long-standing debate whether cytokine signaling has instructive or permissive

effects on lineage commitment (Enver et al., 1998; Metcalf, 1998). On the one hand, observations that differentiation can occur in the absence of lineage-specific cytokine signaling and that ectopic receptor expression can induce proliferation without commitment to the cytokine affiliated pathway (Enver et al., 1998; Lagasse and Weissman, 1997; McArthur et al., 1994; Metcalf, 2008) have been interpreted as a permissive role of cytokine signaling in lineage engagement. On the other hand, examples have been reported where ectopic cytokine signals resulted in lineage conversion and thus supported an instructive model (Kondo et al., 2000; Pawlak et al., 2000).

MafB is a bZip type transcription factor that is highly expressed in mature monocytes and macrophages (Eichmann et al., 1997; Kelly et al., 2000; Sieweke et al., 1996). Overexpression of MafB limits myeloid progenitor proliferation (Tillmanns et al., 2007) and accelerates macrophage differentiation (Gemelli et al., 2006; Kelly et al., 2000) at the expense of other cell fate options (Bakri et al., 2005; Sieweke et al., 1996). Furthermore, MafB-deficient macrophages show increased responsiveness to M-CSF-induced morphological changes (Aziz et al., 2006). M-CSF has been mainly characterized for its lineage-specific effects on monocytes and macrophages (Pixley and Stanley, 2004), but earlier work also suggested that M-CSF can act on primitive stem and precursor cell populations (Kriegler et al., 1994), which upon more detailed characterization were also found to express its receptor (Akashi et al., 2003; Miyamoto et al., 2002).

Here, we report that MafB deficiency specifically sensitized HSC populations to M-CSF-induced cell division, specific upregulation of the early myeloid selector gene *PU.1*, and a dramatically enhanced myeloid-specific repopulation activity that does not affect self-renewal or differentiation into other lineages. Our results point to a role for MafB in the maintenance of a balanced lineage potential of HSCs by selectively restricting myeloid commitment divisions that give rise to PU.1$^+$ progenitors in response to M-CSF signaling. Together, these data suggest that the potential of stem cells to produce differentiated progeny of a specific lineage bias can be subject to control by integrated cytokine/transcription factor circuits, where variation in cell-intrinsic sensitivity limits like those set by MafB can render external cues such as M-CSF instructive.

RESULTS

Myeloid Lineage-Specific Repopulation Advantage of MafB$^{-/-}$ HSCs

Hematopoietic transcription factors with established roles in mature myeloid cells can have important functions in HSCs (Iwasaki and Akashi, 2007; Orford and Scadden, 2008; Orkin and Zon, 2008; Zon, 2008). Here, we found significant expression of the monocyte/macrophage transcription factor MafB in the highly LT-HSC-enriched CD34$^-$Flt3/Flk2$^-$ population of the primitive c-kit$^+$,sca$^+$,lin$^-$ (KSL) bone marrow fraction but not in downstream progenitors such as in Flt3$^+$KSL multipotent progenitors (MPPs) or in committed common myeloid (CMPs) and granulocyte macrophage (GMPs) progenitors (Figures S1A and S1B available online). Since MafB can influence cellular proliferation in other systems and cell-cycle status is an important determinant of stem cell activity, we further investigated the effect of MafB deficiency on HSC proliferation. Cell-cycle analysis consistently revealed an increased rate of cell division for *MafB*$^{-/-}$ cells in highly LT-HSC-enriched populations as defined by two different stringent marker combinations (CD150$^+$ or CD34$^-$ cells in the Flt3$^-$ KSL [KSLF] fraction) but not in other KSL fractions (Figures 1A, 1B, S1C, and S1D). When we further analyzed the consequence of increased HSC proliferation on stem cell activity in competitive reconstitution assays, we observed a significant advantage of *MafB*$^{-/-}$ but not WT HSCs over normal competitors that resulted in an increased contribution of *MafB*$^{-/-}$ cells to the KSL fraction (Figure 1C). Surprisingly, this competitive advantage of *MafB*$^{-/-}$ HSCs differentially affected the repopulation of lymphomyeloid progeny and resulted in striking differences in the contribution to the different lineages of the hematopoietic system. Twelve weeks after reconstitution, myeloid Mac1$^+$ cells in spleen and bone marrow were largely derived from *MafB*$^{-/-}$ donor cells with a 3-fold and 11-fold excess over WT competitors, respectively (Figure 1D). This competitive advantage was maintained long term in the KSL fraction (Figures S2A and SB) and in mature myeloid cells, including F4/80$^+$ macrophages (Figure 1D). Interestingly, however, the repopulation advantage was lineage specific and not observed in lymphoid or erythroid cells. In peripheral lymphoid organs or bone marrow, the original ratio of donor to competitor was maintained in T cells and only slightly increased in B cells (Figure 1D). Since erythroid cells only weakly express the Ly5 surface marker, we analyzed *MafB*$^{-/-}$ donor contribution to Ter119$^+$ erythroid cells in the bone marrow by quantitative genomic PCR. Quantitative PCR of nonfunctional GFP sequences that specifically tag the *MafB* knockout allele (Blanchi et al., 2003) revealed a linear standard curve for defined mixtures of WT and *MafB*$^{-/-}$ cells (Figures 1E and 1F) and a consistent advantage of *MafB*$^{-/-}$ donor cell contribution to Mac-1$^+$ cells but a contribution to the erythroid lineage that reflected the original injected donor to competitor ratio (Figure 1G).

Despite this strong myeloid-biased repopulation advantage, we did not observe an abnormal increase of total KSL (Figures S2C and S2D) or myeloid cells, myeloproliferative disorders, or leukemia (Figure S3), indicating that *MafB*$^{-/-}$ HSCs outcompeted WT cells in the generation of myeloid progeny without transgressing compartment limits.

To further define the efficiency and kinetics of the myeloid specific repopulation advantage, we followed the contribution of *MafB*$^{-/-}$ HSCs to blood leukocytes of mice reconstituted in excess of competitor cells. Whereas at all dilutions *MafB*$^{-/-}$ stem cells continued to stably contribute to T cells at the initially injected ratio over the whole observation period, we observed a progressively increasing contribution of *MafB*$^{-/-}$ Ly5.2 but not WT Ly5.2 cells to myeloid Mac-1-positive leukocytes even at a 10-fold or 50-fold excess of competitor cells (Figure 2A). This became first observable between 4 and 6 weeks after reconstitution, when originally coinjected progenitors have exhausted their life span and peripheral blood cells will be mainly derived from donor stem cells. At 15 weeks after reconstitution with equal initial input, *MafB*$^{-/-}$ cells had almost entirely outcompeted the WT cells, and in the case of a 10-fold initial competitor excess still provided most cells in the Mac-1-positive population.