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Promotive effect of macrophage colony-stimulating factor on long-term engraftment of murine hematopoietic stem cells

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Abstract

Large ex vivo expansion of hematopoietic stem cells (HSCs) sufficient for use in clinical applications has not been achieved, although the influence of some cytokines including SCF, IL-11, Flt3-L, and TPO for this purpose has been reported. We present evidence for an indirect effect of macrophage colony-stimulating factor (M-CSF) on expansion of murine HSCs. Fresh $Lin^{-/low}$ cells were isolated from Ly5.1 mouse bone marrow and cultured with or without M-CSF in the presence of SCF + IL-11 + Flt3-L or SCF + IL-11 + TPO for 6 days. The expanded cells were harvested and transplanted into lethally irradiated Ly5.2 recipients with competitor cells. Culture of $Lin^{-/low}$ cells with M-CSF significantly enhanced long-term engraftment. When the more enriched HSC populations of $Lin^{-/low}$ c-Kit^{+} Sca-1^{+} cells were used as a source of HSCs, such a promotive effect was not observed, in agreement with negative expression of the M-CSF receptor (c-Fms). However, co-culture with $Lin^{-/low}$ c-Fms^{+} resulted in a significant increase of long-term engraftment. These results suggested that M-CSF is an indirect stimulator for ex vivo expansion of HSCs in the presence of SCF, IL-11, Flt3-L, and TPO. These observations provide new directions for ex vivo expansion and insight into new engraftment regulation through M-CSF signaling.

Keywords: Hematopoietic stem cells; Long-term repopulation; M-CSF; c-Fms; Ex vivo expansion

1. Introduction

Hematopoietic stem cells (HSCs) are defined as cells with extensive proliferative potential and capability to differentiate into all hematopoietic lineages. Specific cell-surface marker(s) identifying HSCs are not available and HSCs are evaluated through their unique ability for long-term repopulation (LTR) of all hematopoietic lineages in transplant models [1]. By using this assay system, others and we have shown that the LTR of HSCs is regulated positively by cytokines such as stem cell factor (SCF), interleukin-11 (IL-11), fms-like tyrosine kinase 3-ligand (Flt3-L), and thrombopoietin (TPO) [2–7].

Macrophage colony-stimulating factor (M-CSF) is a lineage-specific hematopoietic regulator that stimulates the survival, proliferation, and differentiation of monocytes/macrophages [8]. The effects of M-CSF are mediated by c-Fms, a high-affinity receptor tyrosine kinase encoded by the *c-fms* proto-oncogene [9].

While previous studies showed that HSCs contain low levels or no mRNA for c-Fms [10–12], we evaluated the influence of M-CSF on their long-term repopulating activity (LTR-activity) in $Lin^{-/low}$ cells. LTR-activity was promoted in an indirect fashion and the presence of

Abbreviations: HSCs, Hematopoietic stem cells; SCF, Stem cell factor; IL-11, Interleukin-11; Flt3-L, Fms-like tyrosine kinase 3-ligand; TPO, Thrombopoietin; M-CSF, Macrophage colony-stimulating factor.

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$Lin^{-/low}$ c-Fms^{+} cells that can respond to M-CSF was found to be important.

2. Results

2.1. Characterization of long-term repopulating assay

A quantitative analysis of HSCs is required for expansion studies and we normalized the LTR-assay system for linearity (Fig. 1). Increasing numbers of Ly5.1-donor bone marrow cells were transplanted into lethally irradiated Ly5.2 recipients with 1×10^6 Ly5.2 competitor cells. After 24 weeks, the donor contribution (ratio of %Ly5.1 to %Ly5.2) was proportional to the number of donor cells with a high correlation coefficient ($R^2 = 0.996$). The linear portion of the curve was used for quantitative analyses.

2.2. Promotive effect of M-CSF on the long-term repopulating activity of HSCs

Since HSCs in bone marrow are enriched in $Lin^{-/low}$ cells [13,14], we used this population as a source of HSCs for the primary expansion study. Several cytokines including SCF, IL-11, Flt3-L, and TPO have been shown to be efficient to some extent for promoting the ex vivo expansion of HSCs [2–7]. To confirm these cytokine effects, we cultured $Lin^{-/low}$ cells with SCF, IL-11, and Flt3-L or TPO for 6 days and examined changes in their LTR-activities (Table 1). Fresh $Lin^{-/low}$ cells (40,000) showed $19.1 \pm 1.9\%$ and $18.5 \pm 1.3\%$ of average engraftment level at 12 and 24 weeks post-transplantation, respectively, suggesting that their bone marrow attained steady state production by 12 weeks after transplantation. $Lin^{-/low}$ cells cultured with the indicated cytokines required longer periods ($\geq$24 weeks) to reconstitute steady state conditions. Therefore, we compared long-term engraftment levels at 24 weeks after transplantation. LTR-activities of $Lin^{-/low}$ increased slightly in cultures containing SCF + IL-11 + Flt3-L and SCF + IL-11 + TPO, but these increases were not significant. The further addition of M-CSF to the cultures resulted in a significant increase of LTR-activity compared with fresh control group. These high engraftment levels were observed even after 32 weeks post-transplantation (data not shown). Addition of 10 ng/mL M-CSF was near optimal since the promotive effect was inhibited slightly at 100 ng/mL M-CSF (data not shown). All mice showed multilineage reconstitution at 24 weeks post-transplantation. An example of a reconstitution with cells cultured in the presence of SCF + IL-11 + Flt3-L + M-CSF is shown in Fig. 2. These results show that M-CSF promotes the LTR-activities of HSCs in the presence of SCF, IL-11, Flt3-L, and TPO.

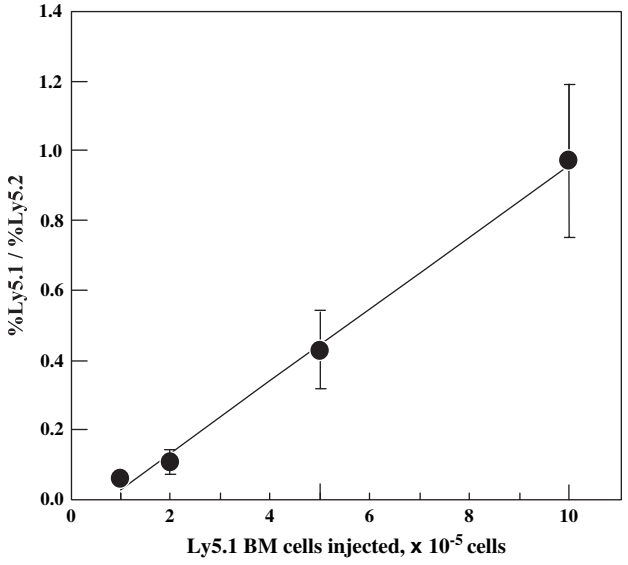

Fig. 1. Linearity of long-term repopulating assay. Bone marrow cells were freshly isolated from Ly5.1 mice and the indicated number transplanted into lethally irradiated mice (Ly5.2) with one million competitor cells. After 24 weeks, peripheral blood was analyzed for the donor contribution by flow cytometry. Data represent mean ± SEM ($n = 4$).

2.3. Expression analysis of M-CSF receptor (c-Fms) on HSCs

HSCs are reportedly negative for c-Fms mRNA expression [10–12]. To confirm that HSCs do not express c-Fms protein on their cell surface, $Lin^{-/low}$ cells were isolated from fresh bone marrow cells and sorted into c-Fms^{-} and c-Fms^{+} sub-populations. Cells positive for the c-Fms were found at low frequency ($\approx$5%) (Fig. 3A). Because the ratio of $Lin^{-/low}$ c-Fms^{-} to $Lin^{-/low}$ c-Fms^{+} cells was approximately 20:1, 100,000 $Lin^{-/low}$ c-Fms^{-} or 5000 $Lin^{-/low}$ c-Fms^{+} cells were transplanted into lethally irradiated mice. Donor contributions were observed only in mice transplanted with the c-Fms^{-} cells, whereas c-Fms^{+} cells had no LTR-activity (Fig. 3B). This result from the phenotypic analysis confirmed that HSCs are negative for the expression of c-Fms in agreement with the previous studies on mRNA levels [10–12]. Taken together, M-CSF appears to be an indirect stimulator for the regulation of HSCs.

2.4. Effect of $Lin^{-/low}$ c-Fms^{+} cells on the expansion of $Lin^{-/low}$ c-Kit^{+} Sca-1^{+} HSCs

When $Lin^{-/low}$ cells were used for the expansion study, the promotive effects of M-CSF were observed as described above. We assessed the effect of M-CSF on the more enriched HSC populations, the $Lin^{-/low}$ c-Kit^{+} Sca-1^{+} cells (Fig. 4A). Fresh $Lin^{-/low}$ c-Kit^{+}