Rapid and Efficient Positive Selection of Hematopoietic Stem and Progenitor Cells from Adult Mouse Bone Marrow

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Introduction

No antigenic marker has yet been shown to be uniquely expressed by murine hematopoietic stem cells (HSCs) or their immediate progeny. However, most of these cells express Sca-1 (Ly6A/E) and c-kit (CD117), neither of which is expressed on the majority of cells in murine hematopoietic tissues, including adult bone marrow (BM). Sca-1 and c-kit are therefore useful as single markers for obtaining enriched populations of primitive hematopoietic cells from this source. Here we describe a preparative immunomagnetic enrichment procedure that allows the rapid and direct selection of c-kit$^+$ or Sca-1$^+$ cells from adult mouse BM using FACS-compatible superparamagnetic nanoparticles. These results demonstrate the utility of this approach for the rapid enrichment of primitive murine hematopoietic cells and show that the functional integrity of repopulating cells *in vivo* is not compromised by the labeling and isolation procedure.

Methods

Bone marrow cells. 10^8 BM cells were used for each protocol. EasySep™ magnetic positive selection was used to select c-kit$^+$ and Sca-1$^+$ cells. Lineage-positive cells were removed (lineage-depleted) using a cocktail of antibodies (CD5, CD11b, CD45R, Gr-1, 7-4, Ter119) and SpinSep™ dense particle sedimentation (StemCell Technologies Inc).

Analysis of enriched cells. The positively selected cells are labeled with the PE-conjugated antibody during the EasySep™ selection protocol and purity can be assessed by flow cytometry directly or after co-staining with either anti-c-kitAPC or anti-Sca-1Alexa647 and a cocktail of lineage markers [(CD3, CD11b, CD45R, Gr-1, Ter119)FITC].

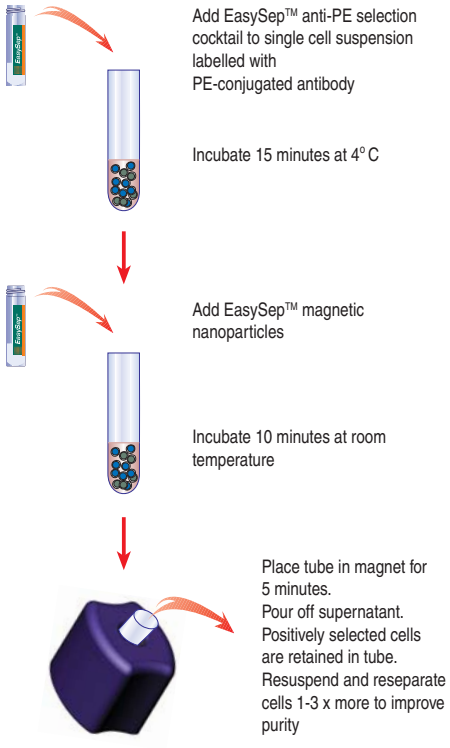
Colony fomring cell (CFC) assay. Pre- and post-enriched BM cells were plated in MethoCult™ GF 3434. Unseparated cells were plated at 10^4/dish. Enriched cells were plated at 1000/dish. Colonies were counted on day 10-12. Total CFCs were defined as BFU-E + CFU-GEMM + CFU-GM.

Competitive repopulating unit (CRU) assay. C57Bl/6 (Ly5.2$^+$) donor cells were injected into groups of irradiated (9 Gy) C57Bl/6/Pep3B (Ly5.1$^+$) recipients together with 10^5 C57Bl/6/Pep3B (Ly5.1$^+$) BM cells. The following cell doses were used per recipient:

A: unseparated (15000, 45000, 90000, 135000);
B: SpinSep™ lineage-depleted (4000, 12000, 24000, 36000);
C: c-kit$^+$ 4x separated (1500, 4500, 9000, 13500);
D: c-kit$^+$ 2x separated (3000, 9000, 18000, 27000);
E: Sca-1$^+$ (2500, 7500, 15000, 22500).

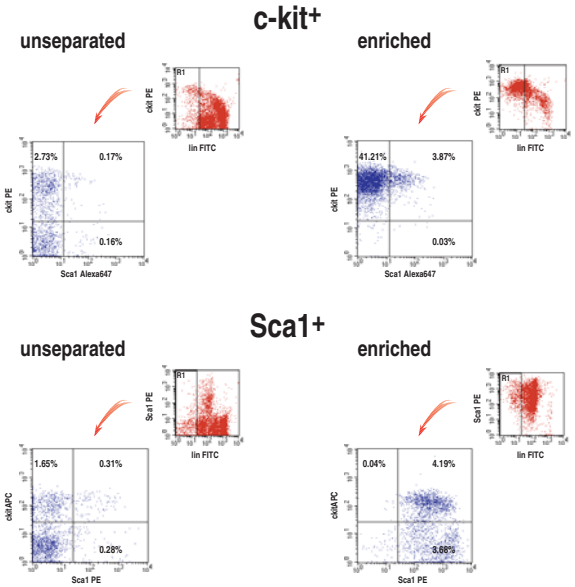
The assay was terminated at 4 months and the presence of donor-derived (Ly5.2$^+$) myeloid (Gr1$^+$) and lymphoid (CD3$^+$/CD19$^+$) WBCs was assessed (reference 1).

Figure 1. EasySep™ procedure for cell selection



Results

Figure 2. Typical FACS plots of unseparated and c-kit$^+$ or Sca-1$^+$ mouse BM cells after EasySep™ immunomagnetic positive selection



Plots are gated on PI$^-$, lineage [(CD3, CD11b, CD45R, Gr-1, Ter119) FITC] negative cells = R1

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Table 1. Purity and recovery of c-kit+ or Sca-1+ mouse BM cells after EasySep™ immunomagnetic positive selection

target cell	phenotype	n	% purity start	% purity enriched	% recovery	fold enrichment	n	% purity enriched	% recovery	fold enrichment
				2x separated*				4x separated*		
c-kit+	c-kit+	6	20±1	68±4	34±7	3.7±0.3	12	92±1	12±2	4.9±0.4
	lin-c-kit+ Sca-1+	3	0.17±0.03	1.5±0.2	58±7	9.2±0.9	3	3.9±0.6	43±5	24±5
Sca-1+	Sca-1+	7	10.8±0.7	70±2	58±4	6.7±0.4	13	94±1	30±2	9±1
	lin-c-kit+ Sca-1+	6	0.43±0.04	3.6±0.4	69±5	7.6±0.8	9	4.3±0.5	37±5	9.9±0.5

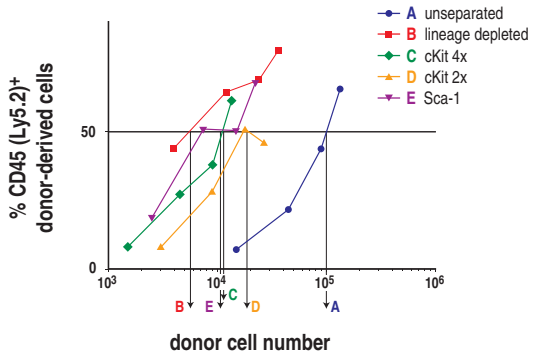
values are means ± 1 sem; n = number of experiments
*2 or 4 rounds of separation

Table 2. Recovery and fold enrichment of CFCs from mouse BM enriched for c-kit+ or Sca-1+ cells using EasySep™ immunomagnetic positive selection

target cell	n	CFC per 1000 cells	total CFC % recovery	fold enrichment	n	CFC per 1000 cells	total CFC % recovery	fold enrichment
unseparated	12	3.4±0.3			12	3.4±0.3		
		2x separated*				4x separated*		
c-kit+	6	32±6	77±6	9±2	11	70±5	57±5	26±4
Sca-1+	6	23±3	54±7	6±1	12	27±3	35±4	10±2

Values are means ± 1 sem; n = number of experiments
*2 or 4 rounds of separation

Figure 3. Percent repopulation in irradiated recipients given unseparated or enriched cell fractions



Engrafted mice showed lymphoid and myeloid repopulation. Data points represent the average % donor-derived repopulation for 3 animals. Number of donor cells required for 50% repopulation (A, B, C, D, E).

Table 3. Frequency, fold enrichment and recovery of CRU from unseparated, lineage depleted, c-kit+ or Sca-1+ selected mouse BM cells

cells	number of donor cells at 50% engrafment level*	CRU frequency**	fold enrichment***	% recovery CRU****
A: unseparated	100, 000	1/16,000	-	-
B; lineage depleted	5,400	1/864	19	13
D: c-kit+ 2x separated	18,000	1/2,880	6	74
C: c-kit+ 4x separated	11,000	1/1,760	9	29
E: Sca-1+	10,500	1/1,680	10	59

Results of one experiment with 3 mice per group. c-kit+ and Sca-1+ cells were selected using EasySep™ magnetic positive selection. Lineage-depleted cells obtained using SpinSep™ dense particle sedimentation.
* from Fig. 3: A, B, C, D and E
**CRU frequency = 1/(number donor-derived cells at 50% repopulation level per 6.25 CRU)
(assuming that 8% repopulation =1 CRU; therefore, 50% repopulation = 6.25 CRU1)
***(CRU frequency in enriched fraction)/(CRU frequency in unseparated fraction)
****[(total number of cells x CRU frequency)$_{\text{post separation}}$ / (total number of cells x CRU frequency)$_{\text{unseparated}}$] x 100

Conclusions

- Hematopoietic stem cells and progenitor cells can be enriched from mouse bone marrow using EasySep™ positive selection of c-kit+ or Sca-1+ cells.
  - No columns required.
  - High cell purity: utilizes the specificity of antibody-mediated selection.
- CFC are enriched over 20-fold with c-kit+ selection and 10-fold with Sca-1+ selection, with good recoveries (Table 2).
- EasySep™ selected c-kit+ and Sca-1+ cells are able to reconstitue multilineage hematopoiesis in irradiated recipients. The selected cells contain ~10 fold higher frequency of repopulating cells than the unseparated cells depending on the chosen protocol.

Together these observations demonstrate that EasySep™ positive selection of c-kit+ and Sca-1+ BM cells is a rapid and efficient means for the enrichment of primitive hematopoietic cells, including repopulating stem cells. This is a useful tool for further fractionation by FACS and for molecular and functional studies.

1. Audet J, Miller CL, Rose-John S, Piret JM, Eaves CJ: Distinct role of gp130 activation in promoting self-renewal divisions by mitogenically stimulated murine hematopoietic stem cells. PNAS 98: 1757-62, 2001