Competitive Bone Marrow Transplantation in C57/BL6 Mice

APPLICATION NOTE

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IN THIS PAPER YOU WILL LEARN

About a method for (mouse) bone marrow transplant based on genetic differences

An associated method to evaluate transplantation results by flow cytometry using the CytoFLEX flow cytometer

Background

Competitive bone marrow transplantation assay measures reconstitution of the blood system adult lineages post-irradiation in transplant recipient mice. The technique hinges on the ability to transplant bone marrow donor cells into a congenic host with normal competitor bone marrow, and is probably the most common and simplest method to determine 'stemness' *in vivo*. In order to distinguish donor from competitor cells upon transplantation, usually competitor mice are congenic and carry the differential B cell antigen originally designated CD45.1/Ly5.1.

Introduction

Hematopoeitic stem cells (HSCs) are cells capable of differentiating into cells of all blood lineages and are essential for maintenance of the blood system. These pluripotent HSCs are capable of self-renewal and regenerate adult blood cells through a hierarchical process generating various multi-potent and lineage-committed intermediate cells.

In order to study the stem niche, researchers must be able to determine the ability of a population of cells to produce adult cell lineages under controlled conditions. A typical strategy employed is to transplant bone marrow, rich in HSCs, into mice whose blood system has been ablated upon sub-lethal irradiation. In these mice both the adult blood cells as well as the stem and progenitor cells will die-off within 2 weeks of irradiation. The transplanted cells will rescue the mice by developing into newly differentiated blood lineages.

HSCs can be subdivided based on their short-term (ST-HSCs), long-term (LT-HSCs) and intermediate-term (IT-HSCS) reconstitution ability [1, 2]. LT-HSCs are determined in most reports by their capacity to generate circulating monocytes, granulocytes, and lymphocytes at time points from 16 to 44 weeks post-transplant. The first analysis of competition by transplanted cells should be done 4-6 weeks post-transplantation.

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Wild-type inbred C57/BL6 mice are typically used in transplant studies given that they carry the *Ptprc*^{b} leukocyte marker (CD45.2/Ly5.2) allele. A congenic strain dubbed C57BL/6-Ly5.1 was developed at the Sloan Kettering Institute by backcrossing SJL mice against wilt-type C57/BL6 mice. These mice carry the *Ptprc*^{b} leukocyte marker (CD45.1/Ly5.1) allele. Using commonly available primary-conjugated antibodies against CD45.1 and CD45.2, researchers can easily identify host and transplanted cells, and determine the contribution of HSCs from either in competition assays. We outline here a simple assay to measure leukocytes in peripheral blood from CD45.2-donor and CD45.1-host mice post-transplantation in irradiated mice.

Procedure

1. On the day of the experiment, one CD45.1 C57/BL6 mouse and one CD45.2 C57/BL6 mouse are sacrificed by preferred method.
2. From these mice, hind limbs are extracted and cleaned.
3. Total bone marrow is flushed from the bone cavity separately with ice cold PBS using a 21 G x 1.5 needle.
4. This is then passed through a 70 µM cell strainer to obtain a single cell suspension.
5. Count the cells under a light microscope using a haemocytometer.
6. Prepare the following mixtures of 2 x 10^{6} cells for transplantation in a total volume of 0.2 mL: (i) CD45.2 and CD45.1 bone marrow cells in a 2:1 ratio, (ii) CD45.1 cells alone and (iii) CD45.2 cells alone
7. Load the 0.2 mL cell suspension into 0.5 mL syringes.
8. The cell suspension is transplanted into irradiated (9.5 Gy) CD45.1 C57/BL6 donor mice by tail vein injection.
9. Following transplantation the donor mice are left to rest for 4 weeks.
10. At 4 weeks post-transplantation, blood is obtained from mice by nicking the tail and collecting approximately 100 µL of peripheral blood into an EDTA lined tube.
11. Collected blood was resuspended in in 10X volume of RBC lysis buffer for 10 minutes (see Note 1).
12. Cells were again resuspended in PBS, with 5 % FBS and kept on ice for 20 minutes
13. Cells were then centrifuged at 400x g for 5 minutes
14. Cells were resuspended in 100 µL staining buffer and incubated at room temperature in the dark for 20 minutes.
15. Cells were centrifuged 400x g for 5 minutes
16. Cells were washed once with PBS
17. Cells were resuspended in 200 µL of PBS
18. Cells were analysed on the Cytoflex flow cytometer.
19. Mouse peripheral blood cells stained CD45.1 FITC and CD45.2 PE were gated for mononuclear cells according to FSC vs. SSC intensity

Materials & Methods

Materials Required:

Centrifuge, microcentrifuge, Gilson pipetteman (P10, P20, P200, P1000), dissection kit, 70 µM Cell Strainer, 21-gauge needles and syringes, 15mL Centrifuge tubes, 1.5 mL microcentrifuge tubes, 2 mL EDTA coated microcentrifuge tubes, scalpel.

Reagents:

1. Red Blood Cell (RBC) lysis buffer: 155 mM Ammonium Chloride (NH_4Cl), 12 mM Sodium Bicarbonate ($NaHCO_3$) and 0.1 mM EDTA were prepared in double distilled H_2O.
2. Phosphate buffered saline (PBS): 8 g NaCl, 0.2 g KCl, 1.44g Na_2HPO_4, 0.24g KH_2PO_4 in 1 L of double distilled H_2O. pH 7.4
3. Staining Buffer: 5 % FBS in PBS. 100 µL staining buffer contains 400 ng CD45.1-FITC and 400ng CD45.2-PE
4. Staining Buffer: 5 % FCS in PBS, made fresh. If not made fresh, an antimicrobial agent such as sodium azide (at a concentration of 0.1 % *v/v*) should be added.

Reagent	Supplier	Order Details
CD45.1-PE	Biolegend	12-0453-81
CD45.2-FITC	Biolegend	11-0454081

Laser	405nm					488nm					638nm		
Fluor	Krome Orange	Pacific Blue	V610	V660	V780	FITC	PE	ECD	PC5.5	PC7	APC	APC AF700	APC AF750
Marker						CD45.2	CD45.1						
Clone						104	A20						

Figure 1.

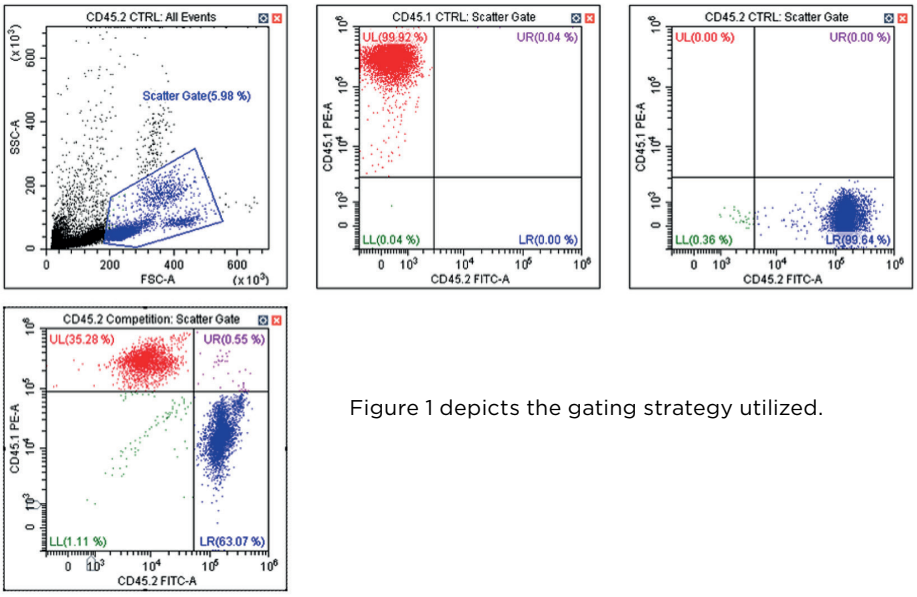

Figure 1 depicts the gating strategy utilized.

Results

Peripheral blood mononuclear cells were gated on the basis of FSC vs. SSC profiles, excluding debris and non-lysed RBCs (Figure 1a). These gated leukocytes were then probed for expression of either the CD45.1 or CD45.2 allele. From the expression of either CD45.1 or CD45.2 we can determine whether a cell originated from the host mouse or from donor bone-marrow. The results show that in mice transplanted whereby CD45.1 bone-marrow cells, as a control, were transplanted back into CD45.1 host mice we only identify CD45.1 cells in the peripheral blood (Figure 1b). The CD45.1 background host mice in which CD45.2 bone marrow cells have been transplanted show a chimeric-blood phenotype, however, at 4 weeks post-transplant (Figure 1c). These mice show an almost 2:1 ratio of CD45.2:CD45.1 leukocytes, as they were transplanted into the mouse originally.

References

1. Li CL, Johnson GR. 1995. Murine hematopoietic stem and progenitor cells: I.Enrichment and biologic characterization. Blood. 1995 Mar 15; 85(6):1472-9.
2. Benveniste P, Frelin C, Janmohamed S, Barbara M, Herrington R, Hyam D, Iscove NN. 2010. Intermediate-term hematopoietic stem cells with extended but time-limited reconstitution potential. Cell Stem Cell. Jan 8;6(1):48-58

Notes

Note 1. It is desirable to remove erythrocytes from spleen mononuclear cell preparations prior to flow cytometry experiments as large numbers of RBCs in the sample can occlude populations of interest. A small number of RBCs remaining in the sample will not prove difficult to gate out however, so partial lysis of RBCs is sufficient and should be optimized depending on the individual experiment being performed.

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