



Interferon Regulatory Factor-2 Regulates Hematopoietic Stem Cells

Atsuko Masumi*

Abstract

Objective: Interferon regulatory factor-2 (IRF-2) is highly expressed in hematopoietic stem cells (HSCs) in mouse bone marrow cells. The population of bone marrow Lin⁻c-Kit⁺Sca-1⁺(KSL) cells is increased in IRF-2^{-/-} mice; however, there are less hematopoietic stem cells due to a reduction of the CD150⁺ population and type I interferon (IFN) signaling. We studied how HSCs lacking IRF-2 differentiate in bone marrow and peripheral blood.

Methods: To study the function of IRF-2 in HSCs, bone marrow cells from IRF-2^{-/-} mice were transplanted into wild-type mice.

Results: Lineage cell population analysis showed that the B220 and CD19 populations from IRF-2^{-/-} mouse bone marrow were enhanced in recipient mice.

Conclusion: Bone marrow cells from IRF-2^{-/-} mice can survive by lineage cell re-population. IRF-2 is important for the long-term repopulation of HSCs through not only the type I IFN response but also via the regulation of lineage-specific cell populations.

Keywords

Interferon regulatory factor-2; Hematopoietic stem cells; Bone marrow; Noncompetitive reconstitution; Lineage cells; Transplantation; Chimerism

Abbreviations:

HSCs: Hematopoietic Stem Cells; IFN: Interferon; IRF: Interferon Regulatory Factor; KO: Knockout; KSL: Lin⁻c-Kit⁺Sca-1⁺; SLAM: Signaling Lymphocytic Activation Molecule; WT: Wild Type.

Introduction

Hematopoiesis is regulated by intrinsic gene regulatory networks, ensuring the rapid production of differentiated blood cells for the immediate generation of definitive hematopoietic stem cells (HSCs). HSCs are thought to be quiescent and are referred to as long-term reconstituting HSCs or dormant HSCs [1-4]. Under certain conditions, such as an infection, HSCs differentiate into progenitor cells with a reduced capacity for self-renewal, and they can be stimulated to differentiate into all cell types found in peripheral blood. Under an inflammatory state, such as during a bacterial or viral infection, an apparent expansion of Lin⁻c-Kit⁺Sca-1⁺ (KSL) cells has

been observed, but the HSCs in the increased KSL population have reduced activity [5,6].

Interferon regulatory factor-2 (IRF-2), a negative regulator of the interferon (IFN) response, is highly expressed in mouse HSCs (KSL) [7]. Previously, an enhanced population of Sca-1⁺ cells and reduced HSC activity in the KSL fraction was detected in bone marrow cells of IRF-2^{-/-} mice [8]. The HSC abnormalities observed in IRF-2^{-/-} mice have been demonstrated to be due to increased type I IFN signaling [9]. In IRF-2^{-/-} mice, the reduced expression of CD150 and enhanced expression of Sca-1 have been detected. In IRF-2^{-/-} IFNAR^{-/-} double knockout (KO) mice, which lack IFN signaling, the expression of CD150 was comparable with that observed in wild-type (WT) mice [8]. However, the activity of HSCs in IRF-2^{-/-} IFN^{-/-} double KO mice was not comparable with that in WT mice, suggesting that IRF-2 partly regulates HSC markers directly [9]. We suggested that HSC-like cells, which may be isolated using cell surface markers distinct from those of KSL cells, are present in the bone marrow cells of IRF-2^{-/-} mice [8].

In the present study, we investigated how HSCs derived from IRF-2^{-/-} mice differentiate into peripheral blood cells.

Experimental

Mice

IRF-2^{-/-} mice were obtained from Professor T. Mak (Amgen Institute, Toronto, Canada) [10]. All mice were maintained in the animal facility of the National Institute of Infectious Diseases under specific pathogen-free conditions and used at 8-20 weeks of age according to the institutional guidelines for animal experimentation.

Antibodies and cytokines

Antibodies against rat IgG2b anti-mouse lineage markers (FITC-CD45.2, APC-CD45.1, APC-c-Kit, FITC-Sca-1, FITC-CD48, APC-CD150, and APC780-CD45.2) were purchased from eBioscience (San Diego, CA).

Isolation of lineage cells and HSCs

Mouse HSCs were purified from bone marrow cells of 8–20-week-old C57BL/6 strain mice. In brief, low-density cells were isolated on Histopaque (Sigma, St. Louis, MO). The cells were stained with an antibody cocktail consisting of PE-conjugated anti-Gr1, anti-Mac1, anti-B220, anti-CD4, and anti-CD8 monoclonal antibodies (BD Biosciences, San Jose, CA). Lineage-positive cells were depleted with anti-PE microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). The remaining lineage-depleted cells were further stained with APC780-CD45.2, FITC-Sca-1, or FITC-CD48 and APC-c-Kit or APC-CD150 antibodies for CD45.2-KSL or CD45.2-signaling lymphocytic activation molecule (SLAM) population analysis. Color analysis and sorting were performed using flow cytometry (JSAN Bay Bioscience, Kobe, Japan).

Noncompetitive reconstitution and chimerism analysis

Noncompetitive bone marrow transplants were performed by the intravenous injection of CD45.2 donor whole bone marrow cells

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(1.0×10^7 , 2.0×10^6 , or 1.0×10^5 cells) from either 8–12-week-old IRF-2 KO or WT controls (C57BL/6) into CD45.1 WT C57BL/6 recipients that had been lethally irradiated with a dose of 9.5 Gy. After transplantation, peripheral blood cells or bone marrow cells were isolated and chimerism was analyzed by staining with FITC-CD45.1 and APC-CD45.2 antibodies.

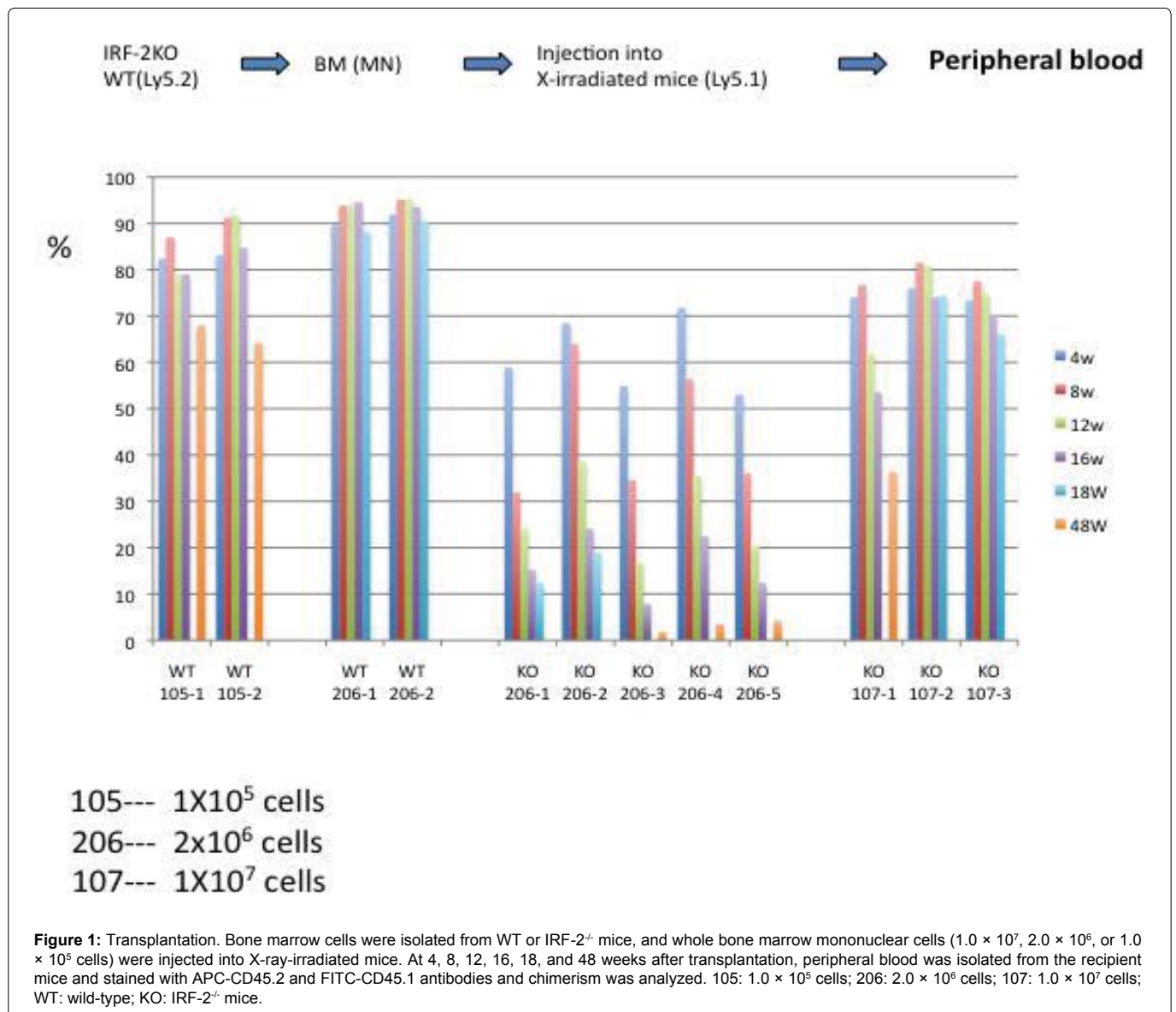
Results

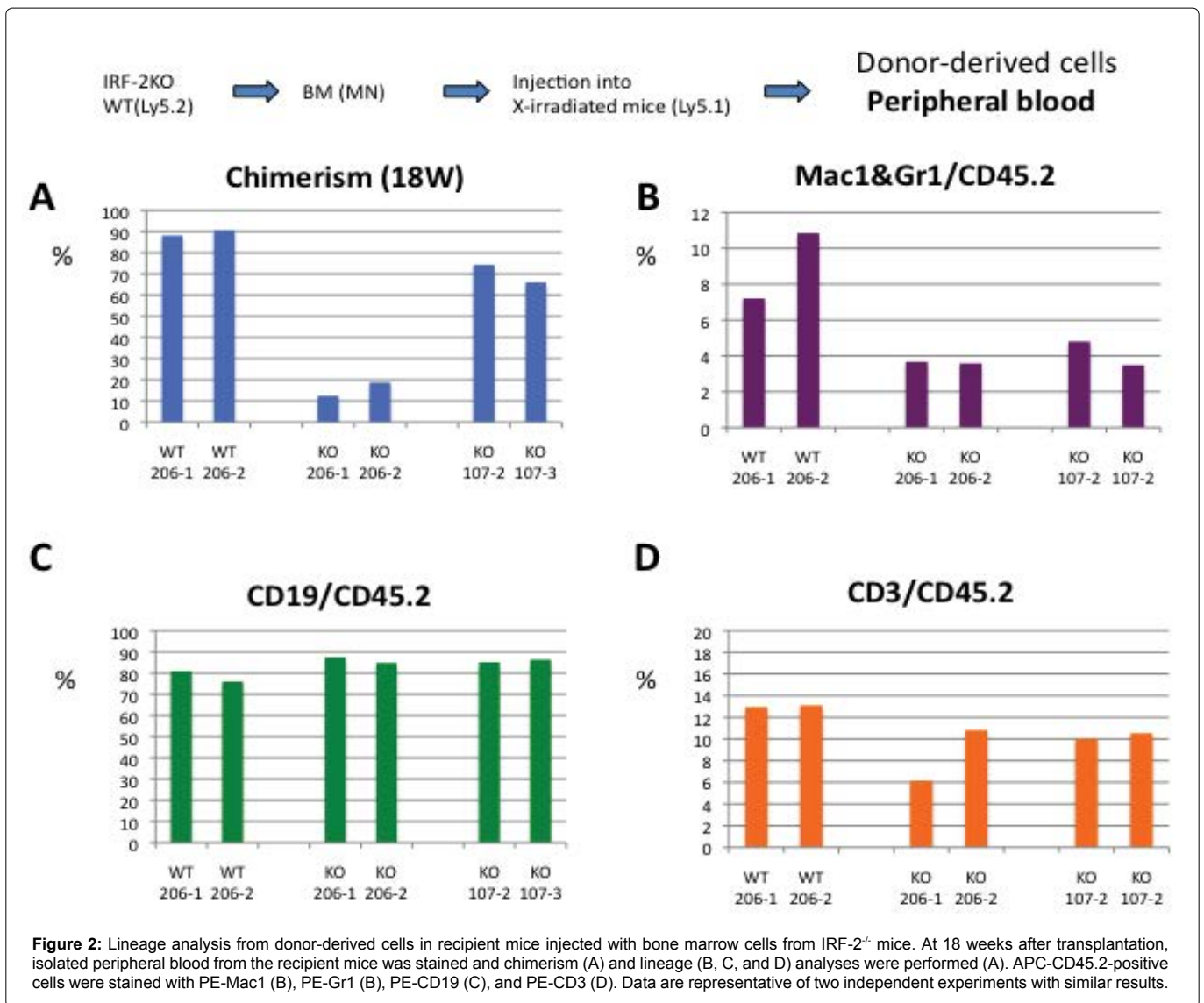
To examine how bone marrow HSCs from IRF-2^{-/-} mice differentiate, whole bone marrow cells were injected into recipient mice and noncompetitive transplant assays were performed. Transplantation of 1.0×10^5 WT bone marrow cells is normally sufficient to rescue and fully repopulate the hematopoietic system of lethally irradiated recipients. However, this dose of cells from IRF-2 KO donors failed to rescue any recipients from lethal irradiation (Figure 1), indicating the impaired self-renewal of HSCs in whole bone marrow cells derived from IRF-2^{-/-} mice. A dose of 2.0×10^6 cells from IRF-2^{-/-} mice rescued the recipients from lethal irradiation,

although the engraftment efficacy was poorer than that of WT cells at 1 month after transplantation (Figure 1). A dose of 1.0×10^7 cells is normally sufficient to rescue and fully repopulate the hematopoietic system of some lethally irradiated recipients.

At 18 weeks after transplantation, we analyzed the lineage populations of donor-derived cells from peripheral blood. Donor-derived peripheral blood analysis showed a reduction of the Mac1/Gr1 and CD3 populations, but not the CD19 population (Figure 2). At 20 weeks after transplantation, KSL and Lin⁻ CD48⁻ CD150⁺ populations from donor bone marrow cells were analyzed in recipient mice injected with 2.0×10^6 or 1.0×10^7 cells from IRF-2^{-/-} mice. The ratio of the populations of these cells was enhanced in recipient mice injected with cells from IRF-2^{-/-} mice compared with recipient mice injected with cells from WT mice (Figure 3).

We investigated the lineage populations in bone marrow cells from recipient mice at 20 weeks after injection with bone marrow cells from IRF-2^{-/-} mice. As shown in (Figure 4), the CD19 and B220 cell





populations were enriched in mice injected with 2.0×10^6 or 1.0×10^7 bone marrow cells from IRF-2^{-/-} mice, although Mac1⁺ cells were enriched in recipient mice injected with bone marrow cells from WT mice.

Discussion

In this study, the function of bone marrow cells from IRF-2^{-/-} mice was investigated. The injection of a large number of bone marrow cells ($2.0 \times 10^6 - 1.0 \times 10^7$) is, in part, normally sufficient to rescue and fully repopulate the hematopoietic system of some lethally irradiated recipients. The populations of lineage cells from donor-derived cells in recipient peripheral blood were different between WT donors and IRF-2^{-/-} donors. A reduction of the Mac1/Gr1 and CD3 populations and slightly higher CD19 population were detected in peripheral blood cells from KO donors. An increase in Mac1⁺ and Gr1⁺ cells and a decrease in B220⁺ cells have been detected in the bone marrow and spleen of IRF-2^{-/-} mice [8]. A reduction of the Mac1/Gr1 population in recipient peripheral blood cells leads to an increase in these populations in bone marrow cells of IRF-2^{-/-} mice. In Table 1, Mac1/Gr1 and CD19 or B220 population in peripheral blood or bone marrow from IRF-2^{-/-} mice or IRF-2^{-/-} donor-derived cells were

compared with those of WT mice or WT donor-derived cells. The populations of lineage cells in bone marrow were different between IRF-2^{-/-} mice and recipient mice injected with IRF-2^{-/-} donors. In bone marrow cells of IRF-2^{-/-} mice, the impaired function of HSCs might be maintained by the regulation of lineage cell populations in bone marrow cells.

Conclusion

In summary, we found that the blood cell populations of IRF-2^{-/-} mice are rearranged to maintain blood homeostasis. In a clinical field, IRF-2 serves as an essential regulator of HSCs and hematopoiesis including stem cell homeostasis.

Ethics approval and consent of participate

All animal protocols were approved by the Institutional Animal Care and Use Committees of the National Institute of Infectious Diseases.

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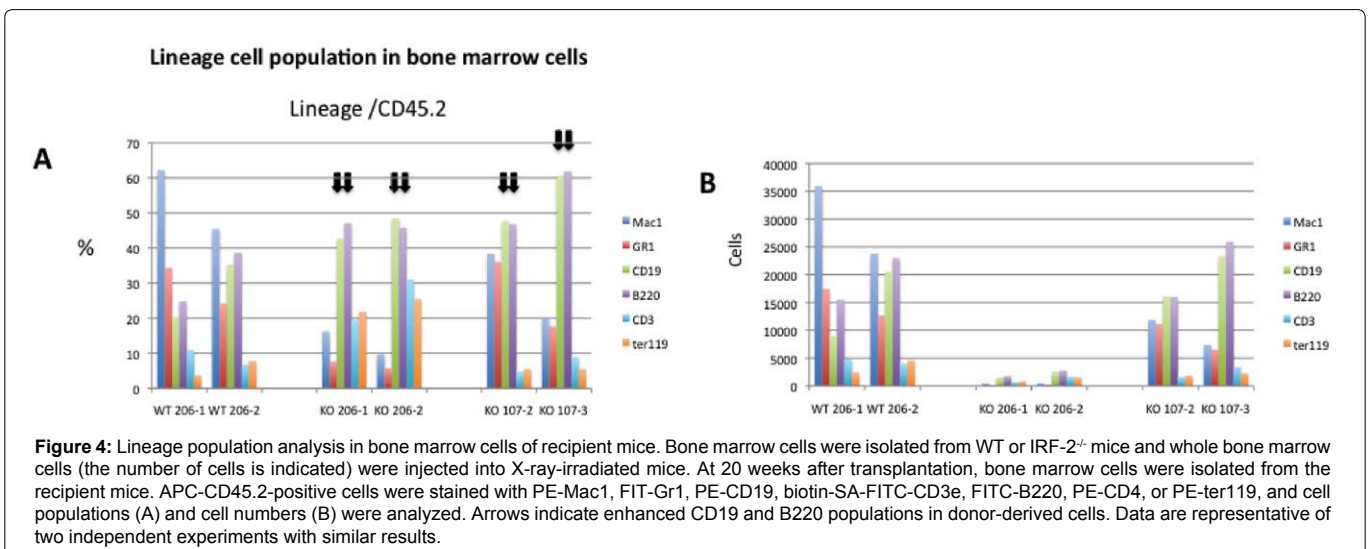
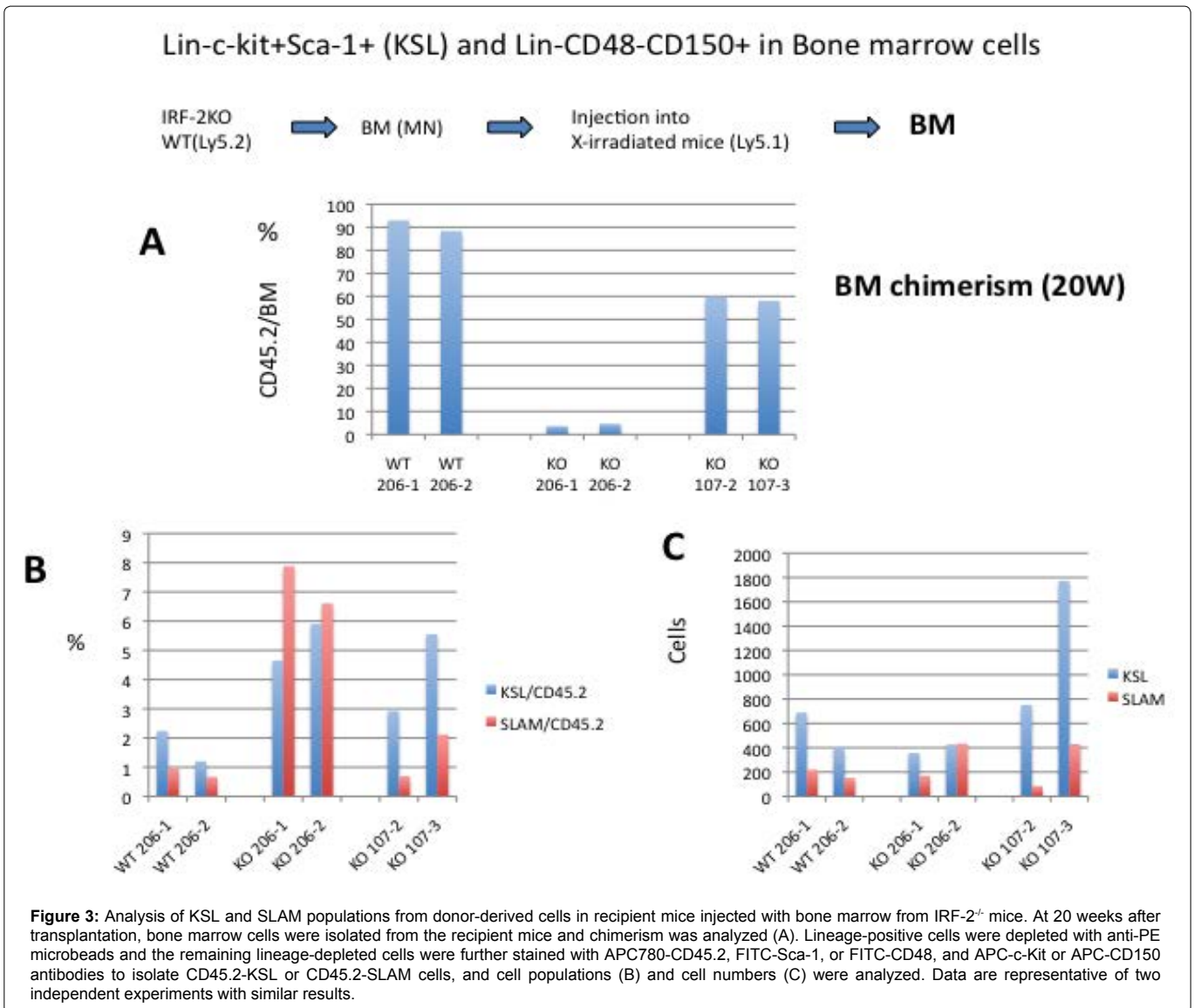


Table 1: The population in peripheral blood or bone marrow from IRF-2^{-/-} mice and IRF-2^{-/-} donor-derived cells were compared with those of WT mice and WT donor-derived cells each other. IRF-2^{-/-}: IRF-2^{-/-} mice, IRF-2^{-/-} donors: recipient mice injected with IRF-2^{-/-} bone marrow cells.

	Mac1/ Gr1		CD19 or B220	
	Peripheral blood	Bone marrow	Peripheral blood	Bone marrow
IRF-2 ^{-/-} [8]	No change	Increase	No change	Increase
IRF-2 ^{-/-} Donors	Decrease	Decrease	Slightly Increase	Increase

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