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## PGE<sub>2</sub> pulsing of murine bone marrow cells reduces migration of daughter monocytes/macrophages in vitro and in vivo

Terence A. McGonigle<sup>a</sup>, Amy R. Dwyer<sup>b</sup>, Eloise L. Greenland<sup>b</sup>, Naomi M. Scott<sup>a</sup>, Kevin N. Keane<sup>c</sup>, Philip Newsholme<sup>c</sup>, Helen S. Goodridge<sup>d</sup>, Leonard I. Zon<sup>e,f</sup>, Fiona J. Pixley<sup>b</sup>, and Prue H. Hart<sup>a</sup>

<sup>a</sup>Telethon Kids Institute, University of Western Australia, West Perth, Western Australia, Australia

<sup>b</sup>School of Biomedical Sciences, University of Western Australia, Western Australia, Australia

<sup>c</sup>School of Biomedical Sciences, Curtin Health Innovation Research Institute Biosciences, Curtin University, Perth, Australia

<sup>d</sup>Board of Governors Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA

<sup>e</sup>Stem Cell Program and Division of Hematology/Oncology, Boston Children's Hospital, Harvard Stem Cell Institute, Harvard, Boston, MA

<sup>f</sup>Howard Hughes Medical Institute, Harvard Medical School, Boston, MA

### Abstract

Monocytes/macrophages differentiating from bone marrow (BM) cells pulsed for 2 hours at 37°C with a stabilized derivative of prostaglandin E<sub>2</sub>, 16,16-dimethyl PGE<sub>2</sub> (dmPGE<sub>2</sub>), migrated less efficiently toward a chemoattractant than monocytes/macrophages differentiated from BM cells pulsed with vehicle. To confirm that the effect on BM cells was long lasting and to replicate human BM transplantation, chimeric mice were established with donor BM cells pulsed for 2 hours with dmPGE<sub>2</sub> before injection into marrow-ablated congenic recipient mice. After 12 weeks, when high levels (90%) of engraftment were obtained, regenerated BM-derived monocytes/macrophages differentiating in vitro or in vivo migrated inefficiently toward the chemokines colony-stimulating factor-1 (CSF-1) and chemokine (C-C motif) ligand 2 (CCL2) or thiolglycollate, respectively. Our results reveal long-lasting changes to progenitor cells of monocytes/macrophages by a 2-hour dmPGE<sub>2</sub> pulse that, in turn, limits the migration of their daughter cells to chemoattractants and inflammatory mediators.

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Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), a derivative of arachidonic acid, can regulate immune responsiveness by direct effects on mature immune cells [1]. Myeloid progenitor cells in the bone marrow (BM) are also regulated by a PGE<sub>2</sub>-rich environment. We have demonstrated reduced immunogenic properties of dendritic cells (DCs) differentiated from progenitors in

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Offprint requests to: Prue Hart, PhD, Telethon Kids Institute, University of Western Australia, P.O. Box 855, West Perth, Western Australia 6872, Australia; Prue.Hart@telethonkids.org.au.

#### Conflict of interest

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the BM of mice implanted 3 days prior with subcutaneous pellets releasing PGE<sub>2</sub> [2]. The effects were long-lasting because DCs differentiating from the BM of both 16-week-engrafted PGE<sub>2</sub>-chimeric mice and serial 16-week-engrafted PGE<sub>2</sub>-chimeric mice had similar sustained, poor immunogenic properties, suggesting epigenetic modification of hematopoietic stem and progenitor cells [2]. This hypothesis was further supported by experimentation with the demethylating agent 5-aza 2-deoxycytidine in UV-chimeric mice [3]. We have described previously experiments with indomethacin showing that UV irradiation of skin modulates DC progenitors indirectly in BM via the intermediate PGE<sub>2</sub> [4].

Pulsing of human cord blood donor cells with a stabilized derivative of PGE<sub>2</sub>, 16,16-dimethyl-PGE<sub>2</sub> (dmPGE<sub>2</sub>) prior to infusion is safe [5,6]. We now report that a similar 2-hour pulse of murine BM cells with dmPGE<sub>2</sub> reduced the migration capabilities of monocytes/macrophages differentiating from those BM cells significantly both in vitro and in vivo.

## Methods

### Pulsing BM cells with dmPGE<sub>2</sub>

All experiments complied with the ARRIVE guidelines and were performed with the approval of the Telethon Kids Institute Animal Ethics Committee. As shown previously for umbilical cord cell preparations [6], freshly isolated BM cell suspensions (10<sup>7</sup> cell/mL) [2–4] were pulsed for 2 hours at 37°C with 10 μmol/L dmPGE<sub>2</sub> (Sigma-Aldrich, St. Louis, MO). dmPGE<sub>2</sub> was obtained dissolved in methyl acetate, which was evaporated off at 4°C in argon, an inert gas, as per the manufacturer's instructions; the remaining precipitate was then dissolved in RPMI-1640 supplemented with 10% FCS (HyClone, GE Health Care Life Sciences, Logan, UT). As a control, cells were incubated with an equivalent volume of RPMI-1640 with 10% FCS for 2 hours at 37°C.

### Generating dmPGE<sub>2</sub>-chimeric mice

BM cells were isolated from naive congenic B6.SJL-Ptprca mice (CD45.1 alloantigen) and then incubated at 37°C for 2 hours with 10 μmol/L dmPGE<sub>2</sub> or control medium, as described above. Eight-week-old C57BL/6J mice (recipients, CD45.2 alloantigen) were gamma-irradiated (2 × 550 rad) using a <sup>137</sup>Cs source (Gammacell 3000 Elan, MDS Nordion, Ottawa, Canada) before injection of 2 × 10<sup>6</sup> BM cells that had been pulsed with dmPGE<sub>2</sub> (dmPGE<sub>2</sub>-chimeric mice) or control medium (control-chimeric mice).

### Culture of BM cells for differentiation of macrophages and chemotaxis toward CSF-1 and CCL2

Macrophages were differentiated from BM cells (after a dmPGE<sub>2</sub> pulse or after isolation from chimeric mice) as described previously [7–9]. Briefly, BM cells were cultured in RPMI-10 and 0.6 ng/mL (50 IU/mL) CSF-1 (gift from Dr. E.R. Stanley) for 24 h. Nonadherent cells were then collected and cultured with 12 ng/mL CSF-1 for 3 days (replated on day 2), followed by 5 days with 120 ng/mL CSF-1 before use. Adherent cells were uplifted using PBS containing 2 mmol/L ethylenediaminetetraacetic acid (EDTA). BM-differentiated macrophages (2.5 × 10<sup>5</sup>) were seeded, in replicate, into Transwell inserts

with 8  $\mu\text{m}$  pores (BD Biosciences) in 200  $\mu\text{L}$  of RPMI-10 (CSF-1-free or CCL-2-free) and inserts were placed into a 24-well companion plate with complete medium containing 120 ng/mL CSF-1 or 20 ng/mL CCL2, respectively. Although CSF-1 is necessary for macrophage growth and differentiation, it is also a potent chemokine that stimulates macrophage migration [8,9]. CCL2 is a macrophage chemoattractant with a recognized ability to recruit monocytes to sites of inflammation [10]. The macrophages migrated for 5 hours (37°C, 5% CO<sub>2</sub>) before fixation of inserts in 4% paraformaldehyde, staining for 5 min with NucBlue-fixed cell stain (Life Technologies, Australia), and imaging by confocal microscopy. Migrated cells were counted for 10 representative fields per insert at 20  $\times$  magnification.

### Assay of macrophage migration into the peritoneal cavity

The migration capabilities of monocytes/macrophages into the peritoneal cavity of control-chimeric and dmPGE<sub>2</sub>-chimeric mice were examined by intraperitoneal injection of 1 mL of thioglycollate (3.8% Medium Brewer Modified, Becton Dickinson). After 3 days, the peritoneal cavity was washed out with saline. The harvested cells were counted and identified objectively [11].

### Statistical analyses

There were always at least three independent mice or cell populations per group, with differences judged significantly different ( $p < 0.05$ ) using an unpaired *t* test.

## Results and discussion

Pulsing of freshly isolated BM cells for 2 hours with dmPGE<sub>2</sub> did not alter the rate of growth, proliferation, or differentiation of macrophages after 9 days in culture with CSF-1 (Fig. 1A). Because of our protocol of removing adherent (differentiated) cells after the first 24 hours in culture, differentiation of macrophages from progenitor cells was guaranteed [7,8]. All cells at the end of culture were phenotypically similar (>98% CD11b<sup>+</sup>F4/80<sup>+</sup>). However, those macrophages differentiated from dmPGE<sub>2</sub>-pulsed BM cells migrated less efficiently toward CSF-1 than those differentiated from BM cells pulsed with control medium (Fig. 1B). Reduced expression of the CSF-1 receptor (CSF-1R), also known as CD115, was not responsible (Fig. 1C). Macrophages differentiated from the BM of control-chimeric and dmPGE<sub>2</sub>-chimeric mice were then analyzed to examine the robustness of the dmPGE<sub>2</sub> effect. The initial yield and the rate of growth, proliferation, and differentiation of macrophages after 9 days in culture with CSF-1 were not significantly different between BM cells from control-chimeric and dmPGE<sub>2</sub>-chimeric mice (Fig. 1D). However, reduced migration toward CSF-1 (Fig. 1E) and CCL2 (Fig. 1G) by macrophages differentiated from the BM of dmPGE<sub>2</sub>-chimeric mice was found consistently. These differences were not due to reduced expression of the chemokine receptors CSF-1R and CCR2 (Figs. 1F and 1H).

To exclude any artifact due to macrophage differentiation in vitro, accumulation of monocyte-derived macrophages after thioglycollate injection into the peritoneal cavity of control-chimeric and dmPGE<sub>2</sub>-chimeric mice was examined. For reasons that remain unclear, responses in 4-week chimeric mice were poor, with few macrophages accumulating

in the peritoneal cavity. After 7 and 12 weeks of engraftment, the number of cells accumulating in the peritoneal cavity of dmPGE<sub>2</sub>-chimeric mice was significantly reduced compared with control-chimeric mice (Fig. 2A). There were significantly fewer total macrophages (F4/80<sup>+</sup>CD11b<sup>+</sup>), elicited macrophages (F4/80<sup>lo/int</sup>CD11b<sup>+</sup>), DCs (F4/80<sup>-</sup>CD11c<sup>+</sup>), neutrophils (F4/80<sup>-</sup>CD11b<sup>lo</sup>Gr1<sup>+</sup>), and eosinophils (F4/80<sup>lo</sup>CD11b<sup>lo</sup>) (Fig. 2B–2D). Expression of the CSF-1R (CD115), CCR2 (CD193), CCR7 (CD197), and MHC II was not different for the elicited macrophages harvested from the peritoneal cavity of the two chimeric mouse types (data not shown).

The actions of PGE<sub>2</sub> are both microenvironment and concentration dependent [12]. PGE<sub>2</sub> is proinflammatory at early stages of inflammation [1]. It can also play an anti-inflammatory role [13,14] by downregulating systemic inflammation [15] and protecting vascular function [16]. This study suggests a direct and long-lasting effect of dmPGE<sub>2</sub> on monocyte/macrophage progenitor cells in the BM. Our study suggests that a 2-hour incubation with dmPGE<sub>2</sub> is sufficient to modify BM stem and progenitor cells, resulting in reduced migration capabilities of their monocyte/macrophage progeny.

In patients with leukemia or lymphoma, dmPGE<sub>2</sub>-pulsed, nonfractionated umbilical cord cells demonstrated increased expression of CXCR4 [5,17], surviving, and cyclin D, and reduced expression of caspase 3 in hematopoietic stem cells [5,6]. In fact, multilineage hematopoiesis has been sustained in patients for 27 months after transplantation with dmPGE<sub>2</sub>-pulsed umbilical cord cells [18]. In our murine study, dmPGE<sub>2</sub>-induced increases in the proliferation and differentiation of BM-macrophages were not observed in vitro (Fig. 1A and 1D) and there were no increases in the rate of donor cell engraftment in the BM, spleen, and lymph nodes of the dmPGE<sub>2</sub>-chimeric mice (Table 1). This was likely because the number of stem and progenitor cells transferred into the gamma-irradiated mice was not rate limiting [19,20]. The outcomes of our study should be further examined to assist patients undergoing hematopoietic stem cell transplantation, particularly because the dmPGE<sub>2</sub> pulse is very easy to administer. The safety of such pulsing has been confirmed previously [6]. This study highlights the potential of a short and simple pulse of donor BM cells with dmPGE<sub>2</sub> to limit the development of macrophage-driven pathologies. Based on the outcomes of the present study, a dmPGE<sub>2</sub> pulse of donor cells should be tested in future experimentation with models of chronic graft-versus-host disease, a condition mediated, at least in part, by CSF-1-dependent donor-derived macrophages [21].

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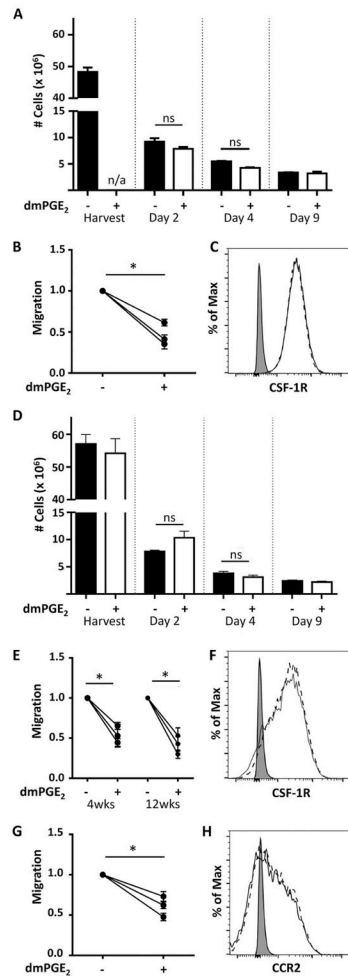
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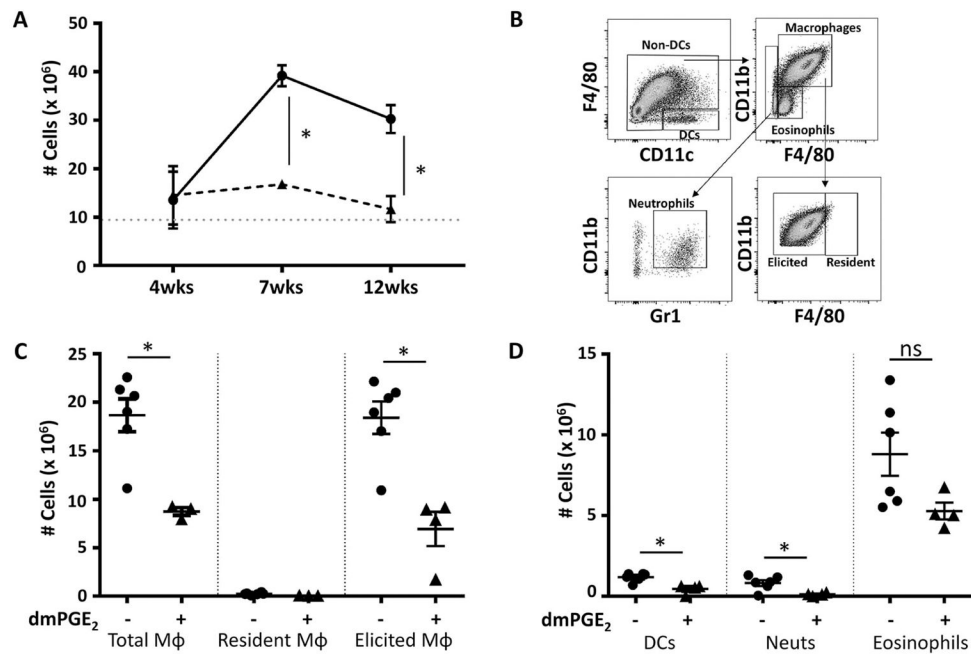
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**Figure 1.**

Similar yields from BM cells in culture but reduced migration toward chemoattractants by macrophages differentiated for 9 days from dmPGE<sub>2</sub>-pulsed BM cells or BM of dmPGE<sub>2</sub>-chimeric mice. **(A)** Cell yields from the BM of naive mice and after their culture for 2, 4, and 9 days (control medium-pulsed cells, solid bars; dmPGE<sub>2</sub>-pulsed cells, open bars). n/a = not applicable. **(B)** Migration response to CSF-1 (120 ng/mL) by macrophages differentiated from BM cells from three mice pulsed at day 0 for 2 hours with dmPGE<sub>2</sub> or control medium. **(C)** Expression of CSF-1R (CD115) by macrophages differentiated from BM cells pulsed with dmPGE<sub>2</sub> (broken line) or control medium (solid line). **(D)** Cell yields from the BM of control-chimeric and dmPGE<sub>2</sub>-chimeric mice and after their culture for 2, 4, and 9 days (control-chimeric mice, solid bars; dmPGE<sub>2</sub>-chimeric mice, open bars). In **(A)** and **(D)** (mean ± SEM for three mice/group with cells from each mouse cultured separately), freshly isolated BM cells were seeded at 10<sup>7</sup> cells/dish. After 2 days in culture, recovered cells were seeded at 2 × 10<sup>6</sup>/dish and, after 4 days in culture, at 10<sup>6</sup>/dish. ns = not significant. After culture for 2, 4, and 9 days, macrophages were lifted with PBS containing 2 mmol/L EDTA. **(E)** Migration response to CSF-1 (120 ng/mL) by macrophages differentiated from the BM of control-chimeric and dmPGE<sub>2</sub>-chimeric mice harvested 4 and 12 weeks after establishment of the chimeric mice. **(F)** Expression of CSF-1R (CD115) by macrophages

differentiated from the BM of 12-week-engrafted dmPGE<sub>2</sub>-chimeric (broken line) and control-chimeric mice (solid line). **(G)** Migration response to CCL2 (20 ng/mL) by macrophages differentiated from the BM of control-chimeric and dmPGE<sub>2</sub>-chimeric mice harvested 12 weeks after establishment of the chimeric mice. **(H)** As for **(F)**, but expression of CCR2 (CD192) by the same cell types. In **(B)**, **(E)**, and **(G)**, the migration by macrophages differentiated from dmPGE<sub>2</sub>-pulsed BM cells **(B)** or BM cells from dmPGE<sub>2</sub>-chimeric mice **(E,G)** is expressed as a proportion of the migration by macrophages differentiated from control-medium-pulsed BM cells or BM cells from control-chimeric mice ( $n =$  three macrophage preparations/group from three independent mice/group). Each point represents the mean migration ( $\pm$ SEM) by macrophages differentiated from the BM of a single mouse (two filter inserts/mouse; 10 images/insert). Asterisk indicates a significant difference in migration ( $p < 0.05$ ) between macrophages differentiated from BM cells pulsed with dmPGE<sub>2</sub> or control medium **(B)** or between macrophages differentiated from the BM of control-chimeric and dmPGE<sub>2</sub>-chimeric mice **(E, G)**. In **(C)**, **(F)**, and **(H)**, the signal from unstained cells (shaded) is shown.

**Figure 2.**

Reduced accumulation of macrophages in the peritoneal cavity of dmPGE<sub>2</sub>-chimeric mice after injection of thioglycollate. Control-chimeric and dmPGE<sub>2</sub>-chimeric mice were injected intraperitoneally with thioglycollate 4, 7, and 12 weeks after the chimeric mice were established. Cells were harvested from the peritoneal cavity after 3 days. **(A)** Total cells harvested from the peritoneal cavity of control-chimeric mice (solid line) and dmPGE<sub>2</sub>-chimeric mice (broken line). The dotted horizontal shows the number of cells harvested from chimeric mice injected with an equal volume of saline. Data are shown as mean ± SEM ( $n = 6$  mice/group). **(B)** Gating strategy [11] for identification of cell types **(C, D)** harvested from the peritoneal cavity 3 days after thioglycollate injection of 12-week-engrafted chimeric mice. **(C)** Number of total, resident, and elicited peritoneal macrophages. **(D)** Number of DCs, neutrophils (Neuts), and eosinophils. For **(C)** and **(D)**, data are shown as mean ± SEM ( $n = 6$  mice/group for control-chimeric mice;  $n = 4$  mice/group for dmPGE<sub>2</sub>-chimeric mice). Asterisk indicates a significant difference ( $p < 0.05$ ) between cells harvested from control-chimeric and dmPGE<sub>2</sub>-chimeric mice. ns = not significant.



**Table 1**

Engraftment of CD45.1<sup>+</sup> donor cells into the BM, spleen, and lymph nodes of control-chimeric (C-chim) and dmPGE<sub>2</sub>-chimeric (dmPGE<sub>2</sub>-chim) mice

	Weeks reconstituted	BM			Spleen			Lymph nodes		
		WT	C-chim	dmPGE <sub>2</sub> -chim	WT	C-chim	dmPGE <sub>2</sub> -chim	WT	C-chim	dmPGE <sub>2</sub> -chim
Total cells ( $\times 10^6$ )	4	60.4 $\pm$ 6.8	46.2 $\pm$ 6.2	49.6 $\pm$ 0.8	103.2 $\pm$ 4.0	64.4 $\pm$ 6.0	55.4 $\pm$ 1.0	-	-	-
	12	62.0 $\pm$ 11.8	50.5 $\pm$ 1.6	46.7 $\pm$ 4.7	74.5 $\pm$ 6.5	70.1 $\pm$ 5.4	80.4 $\pm$ 6.0	-	-	-
%CD19 <sup>+</sup> B220 <sup>+</sup>	12	23.1 $\pm$ 3.0	28.4 $\pm$ 1.8	27.6 $\pm$ 1.4	45.5 $\pm$ 3.5	49.8 $\pm$ 1.9	50.9 $\pm$ 1.7	32.7 $\pm$ 2.6	32.0 $\pm$ 2.8	30.5 $\pm$ 2.9
% CD45.1		0.05 $\pm$ 0.0	97.8 $\pm$ 0.6	98.4 $\pm$ 0.1	0.1 $\pm$ 0.0	99.7 $\pm$ 0.1	99.7 $\pm$ 0.1	0.1 $\pm$ 0.1	99.7 $\pm$ 0.1	99.6 $\pm$ 0.1
%CD3 <sup>+</sup> CD4 <sup>+</sup>	12	1.0 $\pm$ 0.2	1.0 $\pm$ 0.1	0.7 $\pm$ 0.1	11.6 $\pm$ 1.2	13.4 $\pm$ 2.0	9.9 $\pm$ 1.0	21.2 $\pm$ 4.8	33.4 $\pm$ 2.3	33.3 $\pm$ 2.2
% CD45.1		0.1 $\pm$ 0.0	84.9 $\pm$ 2.6	87.6 $\pm$ 3.2	0.1 $\pm$ 0.0	92.1 $\pm$ 1.1	88.9 $\pm$ 1.7	0.1 $\pm$ 0.0	95.1 $\pm$ 1.2	93.0 $\pm$ 0.9
%CD3 <sup>+</sup> CD4 <sup>-</sup>	12	2.5 $\pm$ 0.7	2.9 $\pm$ 0.1	2.1 $\pm$ 0.2	12.1 $\pm$ 0.1	8.8 $\pm$ 0.8	7.4 $\pm$ 0.6	27.7 $\pm$ 6.6	29.5 $\pm$ 0.3	28.1 $\pm$ 0.8
% CD45.1		0.1 $\pm$ 0.0	88.7 $\pm$ 1.6	87.6 $\pm$ 0.8	0.0 $\pm$ 0.0	93.7 $\pm$ 0.2	90.6 $\pm$ 1.2	0.0 $\pm$ 0.0	95.7 $\pm$ 0.3	92.9 $\pm$ 0.6
%F4/80 <sup>+</sup> CD11b <sup>+</sup>	12	8.9 $\pm$ 1.4	13.3 $\pm$ 0.9	14.5 $\pm$ 0.5	1.5 $\pm$ 0.1	2.9 $\pm$ 0.6	3.0 $\pm$ 0.3	1.2 $\pm$ 0.4	1.2 $\pm$ 0.5	0.6 $\pm$ 0.1
% CD45.1		1.5 $\pm$ 0.4	99.8 $\pm$ 0.0	99.8 $\pm$ 0.0	4.6 $\pm$ 0.6	99.1 $\pm$ 0.2	99.1 $\pm$ 0.2	3.5 $\pm$ 0.7	98.9 $\pm$ 0.5	99.7 $\pm$ 0.1
%CD11c <sup>+</sup>	12	2.5 $\pm$ 0.2	2.3 $\pm$ 0.1	2.2 $\pm$ 0.1	3.7 $\pm$ 0.0	3.7 $\pm$ 0.2	4.4 $\pm$ 0.5	3.9 $\pm$ 0.5	4.8 $\pm$ 0.0	3.9 $\pm$ 0.3
% CD45.1		5.5 $\pm$ 1.4	97.3 $\pm$ 0.8	98.1 $\pm$ 0.4	0.4 $\pm$ 0.2	97.0 $\pm$ 0.2	96.0 $\pm$ 0.0	0.4 $\pm$ 0.2	97.7 $\pm$ 0.3	96.5 $\pm$ 0.5

Comparison of cell yields and cell types from wild-type (WT, nonchimeric) mice are shown at 4 and 12 weeks after injection of cells. Data are shown as mean  $\pm$  SEM for three individual mice.