Chapter 3

Interferon-gamma impairs the self-renewal of hematopoietic stem cells

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Abstract
Balancing the processes of hematopoietic stem cell (HSC) differentiation and self-renewal is critical in maintaining life-long supply of blood cells. The bone marrow (BM) produces a stable output of newly generated cells, but immunological stress conditions inducing leukopenia increase the demand for peripheral blood cell supply. How HSC self-renewal and commitment are modulated during immune activation to meet the increased demand for hematopoietic differentiation is largely unknown. It has previously been proposed that the pro-inflammatory cytokine interferon-γ (IFN-γ) plays an important role in this process by promoting HSC proliferation, thereby acting as a positive regulator of HSCs during chronic infection. However, we demonstrate here that IFN-γ rather impairs the maintenance of HSCs by directly reducing their self-renewal capacity both in vitro and in vivo, and that IFN-γ impairs restoration of HSC numbers upon viral infection. We show that IFN-γ reduces thrombopoietin (TPO)-mediated phosphorylation of signal transducer and activator of transcription (STAT)5, which is an important positive regulator of HSC self-renewal. Furthermore, IFN-γ deregulates the expression of the STAT5-mediated cell cycle genes CyclinD1 and p57. These findings demonstrate that IFN-γ is a negative modulator of HSC self-renewal by modifying cytokine responses and expression of genes involved in HSC proliferation. We postulate that the occurrence of BM failure in chronic inflammatory conditions, such as aplastic anemia, HIV and graft-versus-host disease is related to a sustained impairment of HSC self-renewal caused by chronic IFN-γ-signaling in these disorders.

Introduction
Hematopoietic stress conditions, like immune activation, change the hematopoietic output from the BM. Activated lymphocytes have been postulated to influence differentiation of hematopoietic progenitor cells through direct cell-cell interactions mediated by costimulatory molecules. Additionally, activated T cells can suppress the formation of several hematopoietic lineages, such as B cells, erythrocytes and eosinophilic granulocytes through the production of the pro-inflammatory cytokine IFN-γ. BM failure in multiple chronic inflammatory diseases has been associated with elevated IFN-γ levels and the beneficial effect of immune suppressive drugs on hematopoietic function in BM-suppressed patients might result from a reduction in IFN-γ-producing lymphocytes. Which hematopoietic precursors are affected by IFN-γ in these diseases is not known, though the collapse of multiple hematopoietic lineages suggests the failure of a multipotent progenitor. It was recently implied that IFN-γ produced during chronic infection promotes proliferation of long-term repopulating HSCs in vivo and that IFN-γ thereby positively contributes to the maintenance and restoration of blood cell homeostasis upon immunological stress. However, this concept does not explain the negative impact that IFN-γ has on
hematopoiesis in chronic inflammatory diseases. Besides, the mechanism by which IFN-γ can influence HSC self-renewal has not yet been resolved.

**Results**

To address the effect of IFN-γ on HSC function, we cultured highly purified HSCs (Lin-c-Kit+Sca-1+CD48-CD150+) with cytokines supporting both self-renewal and differentiation of HSCs with or without IFN-γ. IFN-γ increased the percentage but not the absolute number of differentiated cells (Lin+), whereas it decreased both the percentage and absolute number of progenitor cells (Lin-c-Kit+) and HSCs (Lin-c-Kit+CD48-CD150⁺; Fig. 1A-B). To assess the functional capacity of these remaining phenotypical HSCs, control and IFN-γ-cultured cells were mixed and transferred to lethally irradiated recipient mice, using the congeneric marker CD45 to distinguish IFN-γ-treated (CD45.1.2) and control donor cells (CD45.1) from host cells (CD45.2; Fig. 1C). These experiments showed long-term, multilineage reconstitution from control cells, but a complete failure from IFN-γ-treated cells (Fig. 1D), thus demonstrating that *in vitro* treatment with IFN-γ reduces HSC numbers both phenotypically and functionally.

To analyze at what level IFN-γ affects HSC maintenance in these cultures, purified HSCs were labeled with Carboxyfluorescein succinimidyl ester (CFSE), cultured for 4 days with IFN-γ and analyzed daily. IFN-γ impaired the expansion of total cells in culture (Fig. 2A) as well as HSC numbers (Fig. 2B), which could not be attributed to increased cell death (data not shown). Based on CFSE-dilution we conclude that the reduction in HSC numbers was due to an inhibitory effect of IFN-γ on HSC proliferation (Fig. 2C-D). Reduction in HSC self-renewal was not due to an increased commitment to differentiate, since IFN-γ did not change the percentage of self-renewing CD48-CD150⁺ HSCs and more committed CD48⁺CD150⁺ progenitors, only their absolute number (Fig. 2E&F). As HSCs are predominantly quiescent in naïve mice, we tested whether IFN-γ influences this non-proliferative state. However, overnight culture with IFNγ did not change expression of the proliferation marker Ki67 (Fig. S1A&B), which is absent in quiescent HSCs², nor the incorporation of 5-bromo-29-deoxyuridine (BrdU) in HSCs and downstream progenitors (Fig. S1C&D). This demonstrates that IFN-γ does not directly affect HSC quiescence or cell cycle entry, which corresponds with the observation that significant differences in HSC proliferation could only be observed after three days of culture (Fig. 2A-D). We therefore conclude that IFN-γ does not affect recruitment of quiescent HSCs into proliferation, but rather inhibits their subsequent self-renewal divisions.

To examine whether IFN-γ has the same effect on HSC function *in vivo*, we infected WT and IFN-γ⁻/⁻ mice with the Armstrong strain of lymphocytic choriomeningitis virus (LCMV). LCMV infection reduces BM-output and induces leukopenia, which is completely dependent on production of type I interferons early after infection¹. LCMV thereby poses significant pressure on the
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Figure 1. IFN-γ reduces HSC maintenance in vitro. HSCs (Lin⁻C-Kit⁺Sca-1⁻CD48⁻CD150⁺) were purified and cultured for 7 days with SCF/TPO/IL-3/IL-6/Flt3-L with or without IFN-γ. (A) Representative plots showing the analysis for progenitors (Lin⁻C-Kit⁺), differentiated cells (Lin⁺), and HSCs (Lin⁻C-Kit⁻CD48⁻CD150⁻) and (B) absolute numbers of these cells. Data represent three independent experiments with 4-5 wells per condition. (C) HSCs from CD45.1 and CD45.1.2 mice were cultured, (CD45.1 HSCs without IFN-γ, CD45.1.2 HSCs with IFN-γ), pooled and analysed and injected into CD45.2 recipient mice. (D) Representative plots showing donor contribution to total white blood cells (WBC) and B, T and myeloid cell lineages in peripheral blood of lethally irradiated CD45.2 recipient mice at 4 weeks after transplantation. (E) Donor contribution to total white blood cells at indicated weeks (n = 8). Experiment was repeated with CD45.1.2 HSCs cultured without IFN-γ and CD45.1 HSCs with IFN-γ with similar results. Mean values ± s.e.m. are shown. *, p < 0.05, **, p < 0.01, ***, p < 0.001. N.D.: not detectable.
Figure 2. IFN-γ impairs self-renewal of HSCs. Purified HSCs (Lin–Kit+CD48–CD150+) were labelled with CFSE and cultured for 4 days with SCF/TPO/IL-3/IL-6/Flt3-L with or without IFN-γ. (A) Expansion of total cells in culture relative to day 1 and (B) expansion of HSCs (Lin–Kit+CD48–CD150+) relative to day 1. (C) Histograms and (D) division index (defined as the average number of divisions that a cell (that was present in the starting population) has undergone) of CFSE-labelled HSCs cultured with or without IFN-γ. (E) Representative plots showing percentage of HSC (Lin–Kit+CD48–CD150+) in cultures and (F) quantification of these data. Histograms and plots are representative of three independent experiments. Graphs represent data pooled from three independent experiments with duplicate cultures. Mean values ± s.e.m. are shown. *, p < 0.05, **, p < 0.01, ***, p < 0.001.
hematopoietic system to restore blood cell homeostasis. As expected, HSC (Lin-c-Kit+CD48-CD150+) numbers were equally reduced in WT and IFN-γ−/− mice at day 4 of infection. However, HSC numbers were restored to normal levels in IFN-γ−/− mice at day 8, while only a slight recovery of WT HSCs was observed at day 12 (Fig. 3A). Impaired recovery of WT HSCs around day 8 paralleled the occurrence of IFN-γ-producing LCMV-specific CD8 T cells (data not shown and 15).

To exclude indirect effects of IFN-γ on HSCs, we generated mixed chimeric mice with BM from both WT and IFN-γR1−/− mice. Two months after transplantation, donor chimerism of HSCs from IFN-γR1−/− mice was higher than from WT mice (Fig. 3B, day 0), suggesting that homeostatic levels of IFN-γ already influence HSC self-renewal. Upon LCMV infection, HSC numbers of both donors dropped equally early after infection, but IFN-γR1−/− HSC recovered much better than their WT counterparts within the same host (Fig. 3C). Importantly, there was no difference in HSC quiescence (Fig. 3D), indicating that the impaired recovery of WT HSCs numbers was due to an IFN-γ-dependent reduction in HSC self-renewal capacity, rather than a decrease in the fraction of cycling HSCs. We therefore conclude that IFN-γ directly reduces HSC self-renewal, both in vitro and in vivo.

Figure 3. IFN-γ inhibits HSC recovery after LCMV infection. (A) WT and IFN-γ−/− mice were infected with LCMV and the number of HSCs (Lin-c-Kit+CD48−CD150+) was measured by flow cytometry at indicated days (n = 3-5). Data represent three independent experiments. Mixed BM-chimeric mice were generated with WT (CD45.1) and IFN-γR1−/− (CD45.2) BM (1:1 ratio), infected with LCMV and (B) donor HSC chimerism, (C) donor HSC numbers and (D) proliferation of donor HSCs were measured at indicated days (n = 3-5). Data are representative for two independent experiments. Mean values ± s.e.m. are shown. *, p < 0.05, **, p < 0.01, ***, p < 0.001.
To investigate the molecular mechanism by which IFN-\(\gamma\) regulates HSC self-renewal, we examined both extrinsic and intrinsic pathways that mediate this process. The cytokine thrombopoietin (TPO) is important for HSC self-renewal, as its receptor, c-mpl, can induce STAT5 phosphorylation and thereby regulate transcription of self-renewal genes\(^4\;16\;17\). Competitive repopulation capacity of STAT5\(^-\) or c-mpl\(^-\) HSCs\(^18\;19\) is severely impaired, while constitutively active STAT5 enhances the self-renewal and repopulation activity of HSCs\(^4\). We found that IFN-\(\gamma\) reduced TPO-mediated phosphorylation of STAT5 in purified HSCs (Fig. 4A,B,C) and fully annihilated TPO-driven HSC expansion (Fig. 4D). IFN-\(\gamma\) induced IRF-1 expression in HSCs, thus confirming direct activation of IFN-\(\gamma\)R signaling, but also induced expression of suppressor of cytokine singling 1 (SOCS1; Fig. 4E), a negative regulator of IFN-\(\gamma\)R signaling through its ability to inhibit STAT1 phosphorylation\(^20\). However, SOCS1 can also inhibit STAT5 phosphorylation\(^20\), which can thereby explain the perturbed TPO-signaling in HSCs by IFN-\(\gamma\).

Next, we investigated the impact of IFN-\(\gamma\) on molecular mediators of HSC proliferation and found that IFN-\(\gamma\) significantly reduced the expression of Cyclin D1 and inhibited TPO-mediated downregulation of cell cycle inhibitor p57 (Fig. 4F). CyclinD1 and p57 are both important mediators of HSC self-renewal and their expression is associated with TPO-signaling and STAT5 expression\(^16\;17\). These findings are also relevant for the in vivo situation, as Cyclin D1 and p57 were significantly down- and upregulated, respectively, in WT HSCs, but not IFN-\(\gamma\)R\(^1-\) HSC from mixed BM chimeric mice upon LCMV infection (Fig. 4G). The combined decrease in STAT5 phosphorylation and changes in expression of these key cell cycle genes provide a molecular explanation on how IFN-\(\gamma\) negatively affects the self-renewal capacity of HSCs both \textit{in vitro} and \textit{in vivo}.

\section*{Discussion}

Our findings challenge a recent report in \textit{Nature}, which concluded that IFN-\(\gamma\) induces HSC proliferation \textit{in vitro} and upon infection with \textit{Mycobacterium Avium}, and caused a concomitant decrease in myeloid progenitors\(^3\). We postulate that part of these conflicting findings can be explained by indirect effects of IFN-\(\gamma\) on other cell types, which we have carefully excluded by treating HSCs with IFN-\(\gamma\) after rather than before cell-sorting and by using WT:IFN-\(\gamma\)R\(^1-\) mixed chimeric mice for \textit{in vivo} studies. Another possible explanation is related to the use of Sca-1 for identification of HSCs and progenitor cells. Sca-1 is an interferon-responsive molecule that is highly upregulated by IFN-\(\gamma\) on all hematopoietic progenitors upon treatment \textit{in vitro} (Fig. S2A) or infection \textit{in vivo} (Fig. S2B). As a consequence, myeloid progenitor cells (normally Sca-1\(^-\)), of which a fraction expresses CD150, become Sca-1\(^+\) and thereby substantially contaminate the HSC pool and reduce the myeloid progenitor fraction (Fig. S2C). We ruled out this contamination by not using Sca-1 when identifying progenitor cells that had been exposed to interferons and by
Interferon-gamma impairs the self-renewal of hematopoietic stem cells excluding CD48+ myeloid progenitors. As shown previously, Sca-1 is not required when HSCs are identified as Lin–c-Kit+CD48–CD150+ cells, both in vivo (Fig. S2D) and in vitro (Fig. S2E). Importantly, Sca-1 upregulation possibly also explains other studies that have reported infection- and interferon-induced increases in HSC numbers.

Figure 4. IFN-γ reduces TPO-mediated STAT5 phosphorylation and affects expression of genes involved in cell cycle regulation. (A) Histogram showing pSTAT5 staining of purified HSCs (Lin–c-Kit+CD48–CD150+) cultured for 24 hours with SCF (shaded histogram), SCF and TPO (bold histogram) or SCF, TPO and IFN-γ (thin histogram). (B) Percentage of cells stained positive for pSTAT5 and (C) geometric mean fluorescence intensity of pSTAT5. Data represent triplicate cultures and are representative for two independent experiments. (D) Purified HSCs were cultured as in Fig. 1 with or without IFN-γ and/or TPO and HSC numbers were measured. Data are representative for two experiments with four wells per condition. (E,F) mRNA expression levels of indicated genes in HSCs cultured for 24 hours with SCF or SCF and TPO with or without IFN-γ. Expression levels are relative to the expression in SCF/TPO cultured HSCs. qPCRs were performed in duplicate and data is pooled from two independent experiments with 1-3 cultures per condition. (G) mRNA expression levels of indicated genes in WT and IFN-γR1–/– HSCs from chimeric mice 8 days after LCMV infection. Expression levels are relative to the expression in WT and IFN-γR1–/– HSCs from naïve WT: IFN-γR1–/– chimeric mice. qPCRs were performed in duplicate of 2-3 pooled samples of purified HSCs. Mean values ± s.d. are shown. *, p < 0.05, **, p < 0.01, ***, p < 0.001.
The observation that IFN-γ directly inhibits HSC self-renewal, but neither HSC quiescence nor differentiation supports previous studies demonstrating that IFN-γ impairs HSC function, without affecting cell cycle entry or viability\textsuperscript{25,26}. Although we did not find evidence that IFN-γ induced differentiation of HSCs \textit{in vitro}, we do not exclude that IFN-γ acts as an inducer of HSC differentiation \textit{in vivo}. Different BM niches regulate the diverse fates of HSCs, like quiescence, self-renewal and differentiation\textsuperscript{27}. IFN-γ signaling might be required to impair HSC self-renewal in order to relocalize them to a microenvironment supporting differentiation. Alternatively, sustained production of IFN-γ might reduce HSC self-renewal in order to prevent exhaustion and loss of HSC activity resulting from excessive HSC proliferation during hematopoietic stress. In both cases, chronic exposure to IFN-γ results in declining HSC numbers and impaired hematopoietic output. It has been postulated that IFN-α induces proliferation of HSCs\textsuperscript{24}, but the use of Sca-1 and frequent omission of CD48 upon HSC identification in that study could also have resulted in an overestimation of HSC numbers. Moreover, part of the observed HSC proliferation upon IFN-α injection was found to be an indirect effect, most likely resulting from feedback mechanisms triggered by the IFN-α-induced leukopenia\textsuperscript{24}. Therefore, it remains unclear if and how IFN-α affects HSC proliferation directly. However, IFN-α might have similar inhibitory effects on HSC self-renewal as IFN-γ, since IFN-α also upregulates SOCS1 and represses megakaryopoiesis by reducing TPO-mediated STAT5 phosphorylation\textsuperscript{28}.

Although temporary inhibition of HSC self-renewal during acute infection will not immediately influence peripheral blood cell numbers, prolongation of this process will threaten the maintenance and/or restoration of blood cell homeostasis. Better understanding of the underlying mechanism is also clinically important, because increased IFN-γ production has been associated with bone marrow-failure in patients with chronic inflammation, such as aplastic anemia, Fanconi anemia, GvHD and HIV\textsuperscript{10-13}. In fact, IFN-γ neutralization improves the \textit{in vitro} capacity of hematopoietic progenitors from patients with aplastic anemia\textsuperscript{29}. Our study suggests that the negative impact of IFN-γ on HSC self-renewal contributes to the impaired hematopoietic function in multiple human diseases and we speculate that repression of IFN-γ signaling could alleviate the hematopoietic suppression in patients with chronic inflammation.

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Materials and Methods

Mice
Wild-type C57BL/6 (CD45.2), C57BL/6.SJL (CD45.1), litters of wild-type C57BL/6 and C57BL/6.SJL (CD45.1.2), and IFN-γ−/− C57BL/6 (CD45.2) were used. IFN-γR1−/− C57BL/6 (CD45.2) (Stock no. 3288) mice were obtained from The Jackson Laboratory. All animals were housed at the animal research institute of the AMC under specific-pathogen-free conditions. Animal experiments were approved by the Animal Ethics Committee and performed in accordance with institutional and national guidelines. For LCMV infection, mice were injected intraperitoneally with 1 x 10^5 PFU of LCMV clone Armstrong in 200 μl PBS.

Transplants
For transplantation of cultured HSCs, CD45.1 and CD45.1.2 cultured cells were pooled, thoroughly washed in PBS, mixed with 2 x 10^5 CD45.2 whole BM cells and injected intravenously into CD45.2 mice lethally irradiated with a split dose of 10 Gy. For WT:IFN-γR1−/− chimeras, 2 x 10^6 WT CD45.1.2 whole BM cells were mixed with 2 x 10^6 IFN-γR1−/− CD45.2 and injected into irradiated WT CD45.1 recipients. Recipient mice received 1.75 gram/liter neomycin in sterile water for the first 3 weeks after transplantation. Chimeric mice were infected with LCMV 2 months after transplantation.

Quantitative real-time PCR
RNA was extracted using Trizol (Invitrogen) and cDNA was made with random hexamers and Superscript II reverse transcriptase (Roche). Quantitative real-time PCR was performed in duplicate with Express SYBR GreenER reagents (Invitrogen) on the StepOnePlus RT-PCR system (Applied Biosystems) and data was normalized using 18S as a reference gene. Primer sequences available on request.

Cultures
For 7-day cultures, 150 HSCs (Lin−c-Kit+c-Sca-1-CD48−CD150+) were sorted directly in 96-well plates cultured in X-VIVO 15 medium (Lonza) supplemented with 5 ng/ml TPO, SCF, IL-3, IL-6 and Flt3-L. IFN-γ (20 ng/ml) was added when indicated. Cells were cultured at 37°C in a humidified incubator at 5% CO2 For STAT5 analysis and RNA collection, HSCs (Lin−c-Kit+c-Sca-1−CD48−CD150+) were cultured for 24 hours with SCF or SCF and TPO in the presence or absence of IFN-γ. All cytokines were obtained from Peprotech. For BrdU incorporation, HSCs (Lin−c-Kit+c-Sca-1−CD34+), ST-HSCs and MPPs (Lin−c-Kit+c-Sca-1−CD34+) and CMPs (Lin−c-Kit+c-Sca-1−CD34−CD16/32low) were purified and cultured for 18 hours with SCF or SCF and IFN-γ for 18
hours. 10 μM BrdU (Sigma) was added for 18 hours (HSCs) or for the last 3 hours of culture (CD34+LKS and CMP). For CFSE-labeling, purified HSCs (Lin–c–Kit–Sca-1–CD48–CD150+) were washed in PBS, labeled with 0.5 μM CFSE (Invitrogen) in PBS for 12 minutes at 37°C, washed and subsequently cultured.

**Flowcytometry**

FACSCanto II and FACSria were used for flow cytometric analysis and cell sorting. For cell sorting, whole BM was stained with biotinylated lineage markers CD4 (GK1.5), CD8α (53-6.7), B220 (RA3-6B2), CD11b (M1/70), Gr1 (RB6-8C5), Ter119 (Ly-76) and IL-7Rα (B12-1) and enriched for progenitors by negative depletion of labeled lineage cells using streptavidin (SA) microbeads and MACS LS-Columns (Miltenyi Biotec). Cells were stained with CD34 (RAM34), Sca-1 (D7), fluorochrome conjugated SA, CD48 (HM-48-1), CD150 (TCF15-12F12.2, Biolegend), CD16/32 (93) and c-Kit. HSCs in all experiments were purified as Lin–c–Kit–Sca-1–CD48–CD150+. For purification of HSCs from LCMV infected chimeric mice cells were stained with additional antibodies against CD45.1 (A20) and CD45.2 (104) and purified as CD45.1+ or CD45.2+ Lin–c–Kit–CD48–CD150+. For the BrdU incorporation assay, cells were purified as Lin–c–Kit–Sca-1–CD34– (HSCs), Lin–c–Kit–Sca-1–CD34+ (ST-HSCs and MPPs) and Lin–c–Kit–Sca-1–CD34+CD16/32low (CMPs). For immunophenotypic characterization of HSCs in culture or in mice, cells were stained as above, with additional antibodies against CD45.1 (A20) and CD45.2 (104) when required, and identified as Lin–c–Kit–CD48–CD150+ in all experiments. Dead cells were excluded by stringent gating on single cells and by using propidium iodide. For staining of phosphorylated STAT5, cultured cells were directly fixed for 10 minutes at 37°C by adding an equal volume of BD Cytofix/Cytoperm buffer (BD Biosciences), chilled on ice for 1 minute, washed, and fixed with -20°C 90% methanol for a least 1 hour. Cells were washed and incubated with pSTAT5 antibody (47, BD Biosciences) at room temperature for 45 minutes. For detection of incorporated BrdU, cells were treated as described elsewhere and BrdU and DNA content was visualized using anti-BrdU (PRB-1) and propidium iodide. All antibodies were obtained from eBioscience, unless indicated otherwise.

**Statistics**

Mean values ± s.d. or s.e.m. are shown. Statistical analysis was performed using a two-tailed Student’s t-test with GraphPad Prism software and significance is indicated by *, p < 0.05, **, p < 0.01, ***, p < 0.001.

**References**


Supplemental figures

Supplemental figure S1. IFNγ does not affect quiescence of HSCs. (A) Representative plots showing gating of HSCs (Lin-c-Kit-CD48-CD150+) and expression of Ki-67 in WT HSCs after overnight culture of total bone marrow with SCF in the presence or absence of IFNγ and (B) quantification of these experiments. Data represent two independent experiments with triplicate cultures. (C) Representative plots showing DNA content and BRDU incorporation in purified HSCs (CD34-LKS), short term-HSCs (ST-HSCs) and multipotent progenitors (MPPs) (CD34+LKS) and common myeloid progenitors (CMPs) (Lin-c-Kit+Sca-1-CD34+CD16/32low) cultured for 18 hours with SCF or SCF and IFN-γ in the presence of BRDU and (D) quantification of these experiments. Plots are representative of three independent experiments. Bar graphs represent data pooled from three independent experiments with 1-3 cultures per condition. *, p < 0.05, **, p < 0.01, ***, p < 0.001.
Supplemental figure S2. IFN-γ induces Sca-1 expression resulting in aberrant HSC identification when CD48 expressing cells are not excluded. (A) Representative plots showing Sca-1 expression on purified LKS cells and myeloid progenitor cells after overnight culture with or without IFN-γ and (B) expression of Sca-1 on Lin-c-Kit+ cells in WT and IFN-γ/- naïve or LCMV-infected mice. (C) Representative plots showing identification of HSCs as Lin-c-Kit+(Sca-1+/-)CD150- or Lin-c-Kit-(Sca-1+/-)CD150+CD34- in WT naïve mice and after infection. HSC gating is shown for Lin-c-Kit+ cells (which equals Lin-c-Kit+Sca-1- cells in infected mice), Lin-c-Kit+Sca-1+ cells and Lin-c-Kit’Sca-1- cells and shows the contamination of HSCs by myeloid progenitors when HSCs are identified as Lin-c-Kit’Sca-1+CD150+ or Lin-c-Kit’Sca-1+CD150+CD34- cells in mice undergoing an infection. Representative plots showing near identical numbers of HSCs when identified as Lin’c-Kit’Sca-1’CD150’CD48- or Lin’c-Kit’Sca-1’CD150’CD48- (D) in mice and (E) after culture. Numbers in all plots indicate percentages of total viable cells.