Stem Cell Reports
Article
OPEN ACCESS

Enhanced Hematopoietic Stem Cell Function Mediates Immune Regeneration following Sex Steroid Blockade

Danika M. Khong,[1,3,5] Jarrod A. Dudakov,[1,2,3,*] Maree V. Hammett,[1] Marc I. Jurblum,[1] Sacha M.L. Khong,[1,6] Gabrielle L. Goldberg,[1] Tomoo Ueno,[1] Lisa Spyroglou,[1] Lauren F. Young,[1] Marcel R.M. van den Brink,[2] Richard L. Boyd,[1,4] and Ann P. Chidgey[1,4,*]

[1]Stem Cells and Immune Regeneration Laboratory, Department of Anatomy and Developmental Biology, Monash University, Clayton, VIC 3800, Australia
[2]Immunology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA
[3]Co-first author
[4]Co-senior author
[5]Present address: Massachusetts General Hospital, Harvard Medical School, Harvard University, Boston, MA 02114, USA
[6]Present address: Department of Surgery, Stanford University, Stanford, CA 94305, USA
*Correspondence: dudakovj@mskcc.org (J.A.D.), ann.chidgey@monash.edu (A.P.C.)
http://dx.doi.org/10.1016/j.stemcr.2015.01.018

SUMMARY

Mechanisms underlying age-related defects within lymphoid-lineages remain poorly understood. We previously reported that sex steroid ablation (SSA) induced lymphoid rejuvenation and enhanced recovery from hematopoietic stem cell (HSC) transplantation (HSCT). We herein show that, mechanistically, SSA induces hematopoietic and lymphoid recovery by functionally enhancing both HSC self-renewal and propensity for lymphoid differentiation through intrinsic molecular changes. Our transcriptome analysis revealed further hematopoietic support through rejuvenation of the bone marrow (BM) microenvironment, with upregulation of key hematopoietic factors and master regulatory factors associated with aging such as Foxo1. These studies provide important cellular and molecular insights into understanding how SSA-induced regeneration of the hematopoietic compartment can underpin recovery of the immune system following damaging cytoablative treatments. These findings support a short-term strategy for clinical use of SSA to enhance the production of lymphoid cells and HSC engraftment, leading to improved outcomes in adult patients undergoing HSCT and immune depletion in general.

INTRODUCTION

One key etiological factor underlying a wide range of diseases is the progressive decline in immune function with age (Dorshkind et al., 2009). At its core is a reduction in lymphopoiesis within the bone marrow (BM) and thymus (Miller and Allman, 2003; Rodewald, 1998), attributed in part to a decrease in the number and function of lymphoid progenitors (Min et al., 2004, 2006). Increasing evidence suggests that intrinsic changes to the earliest hematopoietic stem cells (HSCs) also contribute toward age-related immune degeneration (Geiger et al., 2013). Deficiency in DNA repair, altered DNA methylation patterns, aberrant metabolism and reactive oxygen species, and skewed upregulation of myeloid- (at the expense of lymphoid-) associated genes all contribute to altered HSC function with age (expertly reviewed in Geiger et al., 2013). However, in addition to intrinsic functional changes, extrinsic alterations to the HSC niche also likely to contribute toward the degeneration of HSC function with age (Woolthuis et al., 2011).

Evidence suggests that sex steroids play at least some role in age-related degeneration of lymphopoiesis (Chinn et al., 2012), and we, and others, have previously shown that sex steroid ablation (SSA) is able to rejuvenate aged and immunodepleted BM and thymus, enhance peripheral T and B cell function, and promote immune recovery following hematopoietic stem cell transplantation (HSCT) (Dudakov et al., 2009a; Goldberg et al., 2009; Heng et al., 2005; Sutherland et al., 2005; Velardi et al., 2014). However, the mechanisms underlying SSA-mediated immune regeneration are still unresolved. In particular, the effects of SSA on hematopoietic stem and progenitor cells (HSPCs) are likely to be pertinent given that sex steroids regulate HSC function as well as lymphoid-primed multipotent progenitor (LMPP) cells (Medina et al., 2001; Nakada et al., 2014; Thurmond et al., 2000). In this study, we sought to examine the events upstream of SSA-mediated lymphoid regeneration, focusing on the earliest HSPCs.

RESULTS

SSA Increases the Number of Hematopoietic Stem and Progenitor Cells

Although age-induced reduction in HSC function does not reach its nadir until at least 24 months of age in mice (Morrison et al., 1996), it is clear that significant defects in the capacity for T and B cell differentiation are already evident by middle age (9 months) (Dudakov et al., 2009a; Heng et al., 2005; Sutherland et al., 2005). To determine whether

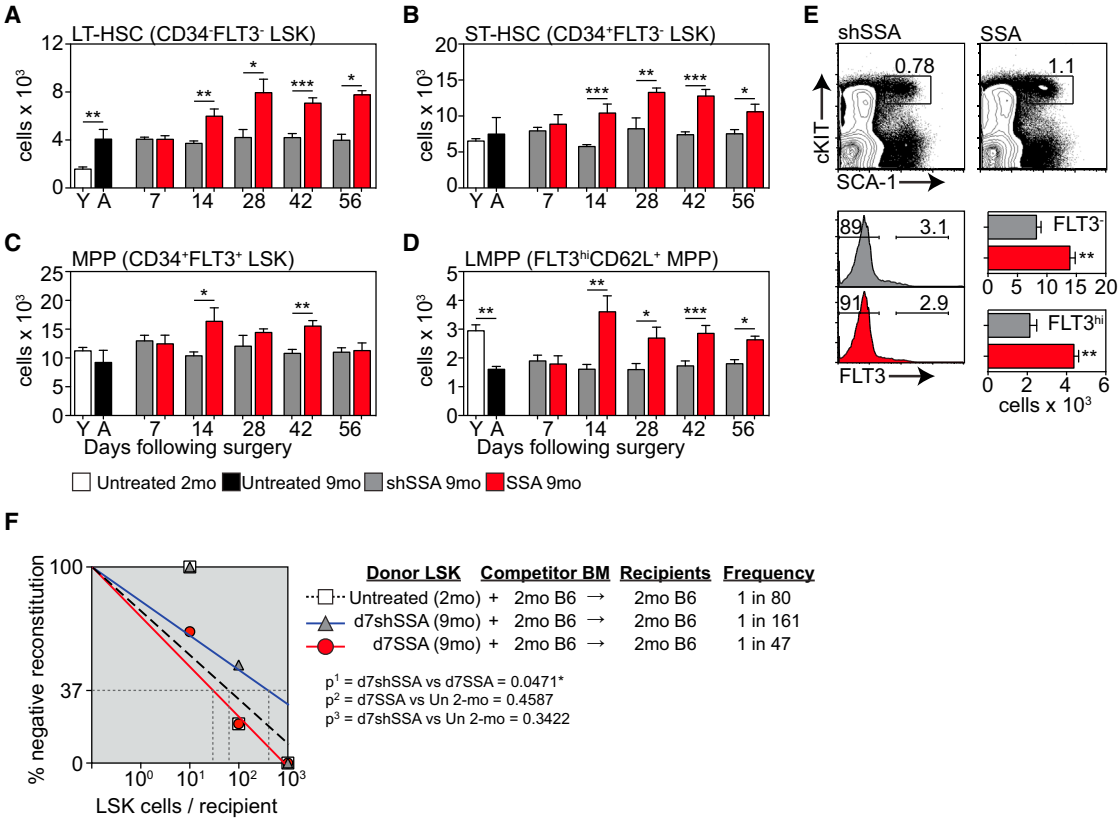

Figure 1. SSA Increases the Number of Multilineage HSCs in Middle-Aged Mice

(A–D) Lin$^-$SCA1$^+$cKIT$^+$ (LSK) BM can be subdivided into populations of LT-HSCs (CD34$^-$FLT3$^-$), ST-HSCs (CD34$^+$FLT3$^-$), and MPPs (CD34$^+$FLT3$^+$). The MPP population can be further fractionated based on FLT3 and CD62L expression for analysis of LMPPs (FLT3^{hi}CD62L$^+$). Absolute number of LT-HSCs (A), ST-HSCs (B), MPPs (C), and LMPPs (D) (n = 5–12/group/time point).

(E) Concatenated flow cytometry plots, gated on Lineage$^-$ cells, and absolute number of FLT3$^-$ LSK cells, 1 year after surgical SSA of 9-month male mice (n = 5/group).

(F) LSK cells were FACS purified from untreated CD45.2$^+$ 2-month; CD45.2$^+$ 9-month mice 7 days following surgical shSSA (d7shSSA); or CD45.2$^+$ 9-month mice 7 days following surgical SSA (d7SSA) (n = 6 recipients/group/dose) and graded doses of cells were transferred into lethally irradiated congenic CD45.1 recipients along with 5 × 10^5 CD45.1$^+$ supporting BM cells. Multilineage reconstitution (>1% B cell, T cell, macrophage, and granulocyte) was analyzed 12 weeks after transplant and the frequency of repopulating cells was calculated by Poisson statistics.

Bar graphs represent mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001. See also Figures S1 and S2.

SSA initiates its impact early in hematopoiesis, we enumerated HSCs by flow cytometry (Figure S1A) at multiple time points after surgical castration of 9-month-old mice. Consistent with previous reports, there was a phenotypic increase in the absolute number of long-term HSCs (LT-HSCs) during aging with a 2-fold increase by middle age (Figure 1A). Following SSA, there was a further increase in the absolute number of LT-HSCs and short-term HSCs (ST-HSCs) from day 14 (d14SSA), which was maintained through to d56SSA compared to sham-SSA (shSSA) control mice (Figures 1A and 1B). While there was no observable impact of age on multipotent progenitors (MPPs), and SSA did not significantly alter their total number (Figure 1C), there was a selective decrease in LMPPs by 9 months, which was reversed following SSA (Figure 1D). This change in HSC number caused by SSA was extremely long-lived with increases in FLT3$^-$ (LT-HSC and ST-HSC) and FLT3^{hi} (LMPPs) still observed 1 year later (Figure 1E).

A defining characteristic of HSC function is the ability to differentiate into multiple lineages. The frequency of multilineage repopulating cells was therefore enumerated using a limiting-dilution competitive repopulation assay (Figure 1F). 10, 100, or 1,000 fluorescence-activated-cell-sorted (FACS) lineage-negative, Sca1$^+$, c-Kit$^+$ (LSK) cells from untreated 2-month mice; 9-month mice 7 days following sham surgery (9-month d7shSSA); or 9-month mice 7 days following surgical SSA (9-month d7SSA) were transferred along with 5 × 10^5 supporting BM cells into