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## Girl Power: Estrogen promotes HSC self-renewal

**Esther Carreras, Ph.D.** and

Immune Receptors of the Innate and Adaptive System Team, Institut d'Investigacions Biomèdiques August Pi i Sunyer, Centre Esther Koplovitz, Rosselló, 149 -153, 08036 Barcelona, Spain, ESCARRER@clinic.ub.es

**Susan Kovats, Ph.D.**

Arthritis & Clinical Immunology Program, Oklahoma Medical Research Foundation, 825 NE 13<sup>th</sup> St., Oklahoma City, OK 73104, Susan-Kovats@omrf.org

### Abstract

Although some hematopoietic cell types are known to respond to sex hormones, hematopoietic stem cells (HSCs) are generally thought to function similarly in both sexes. Recently in *Nature*, Nakada et al. (2014) show that HSCs respond to higher levels of estrogen in females resulting in enhanced self-renewal and increased erythropoiesis.

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Hematopoietic cells express estrogen receptors (ERs) in both females and males, and estrogens and ERs regulate multiple aspects of hematopoiesis and mature immune function (Kovats et al., 2010). An important question is whether the higher levels of estrogens in females, particularly during pregnancy, lead to sex differences in hematopoiesis. Prior work showed that high levels of estrogen in pregnant mice deplete lymphoid-primed multi-potent progenitors, thus decreasing B and T lymphopoiesis (Medina et al. 2001); in contrast, estradiol stimulates dendritic cell differentiation of myeloid progenitors [reviewed in (Kovats et al., 2010)]. However, the potential regulation of HSCs by sex steroids has not been well studied.

Using assays to detect cycling HSCs (defined as lineage-negative Sca-1<sup>+</sup> c-kit<sup>+</sup> (LSK) CD150<sup>+</sup> CD48<sup>-</sup>), including *in vivo* BrdU labeling, Ki-67/propidium iodide staining and turnover of GFP-linked histone H2B, Nakada et al show that HSCs divide more frequently in females than in males. The increased HSC self-renewal in females is reproducible and leads to ~10% more BrdU-labeled HSCs after 10 days. Female multi-potent progenitors (MPPs) also show more frequent divisions.

To determine if endogenous estradiol drives the increased HSC cycling in females, Nakada et al employ ovariectomy and Anastrozole, an inhibitor of the enzyme aromatase required for estradiol biosynthesis. Both treatments reduce HSC cycling to levels seen in males. Exogenous estradiol, injected daily for one week and yielding serum estradiol levels comparable to pregnancy, increased HSC cycling in both females and males. Pregnancy also induced a significant increase in HSC and MPP cycling.

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Correspondence to: Susan Kovats.

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The lower levels of estradiol in unmanipulated males are apparently insufficient to promote increased HSC cycling, suggesting exquisite dose-dependent regulation of HSC self-renewal by estradiol. Alternately, increased HSC division may require a second synergistic signal that is also present at lower levels in males. HSCs do express low levels of progesterone receptor (PR), but injection of progesterone or blockade of PRs with RU486 does not alter HSC cycling. Although androgens modulate lymphopoiesis (Kovats et al., 2010), HSCs do not express androgen receptors and their cycling is not driven by testosterone in females or males. These findings may underlie the superior engraftment observed when human HSCs are transplanted into female immunodeficient mice (Notta et al., 2010).

Estradiol acts via two distinct receptors, the ubiquitous ER $\alpha$  (encoded by *Esr1*) and the more selectively expressed ER $\beta$  (*Esr2*). Nakada et al show that female and male HSCs express *Esr1* but not *Esr2*. ER $\alpha$  is necessary for the estradiol-induced HSC division, as demonstrated by injection of a selective agonist for ER $\alpha$  and use of germline *Esr1*-deficient mice. Deletion of a conditional ER $\alpha$  allele in hematopoietic cells using Vav-Cre and competitive adoptive transfer experiments of ER $\alpha$ -deficient and wild-type HSCs showed that HSC-intrinsic ER $\alpha$  is required for increased HSC cycling in females or in response to ectopic estradiol administration. Rigorous phenotypic definition of HSCs is a relatively recent achievement, and this is the first demonstration that HSCs express ERs and increase their self-renewing divisions in response to estradiol.

Nakada et al find that, in normal females, increased HSC and MPP cycling does not augment their numbers in BM, or lead to elevated BM or spleen cellularity. This unexpected result suggests that the HSCs die more readily, or are mobilized to other tissues. However, female HSCs and MPPs do not enter apoptosis in increased numbers, and the possible migration of HSCs to other tissues was not investigated. A third possibility is that estradiol influences a fraction of dividing female HSCs to rapidly progress to later developmental stages. Indeed, Nakada et al show that normal female mice have an elevated frequency of megakaryocyte-erythroid progenitors (MEPs) but not other lineage-restricted progenitors, suggesting that the increased HSC cycling promotes their progression to erythroid cells. This finding is puzzling since prior work shows that estradiol induces apoptosis in erythroid cell precursors by inhibiting GATA-1; however, it is possible that erythropoiesis is stimulated by the increased apoptosis of BM Ter119<sup>+</sup> cells observed in females. Pregnancy is a distinct case in that increased numbers of HSCs are present in the BM and spleen, which correlates with increases in spleen cellularity, largely due to increased numbers of Ter-119<sup>+</sup> cells, although Mac-1/Gr-1<sup>+</sup> myeloid cells also are elevated. High levels of the pregnancy-specific estradiol may promote this process.

LSK CD150<sup>+</sup> CD48<sup>-</sup> HSCs are functionally heterogeneous (Copley et al., 2012). An unanswered question is whether ER $\alpha$  signaling promotes cycling of all HSC subsets. One intriguing possibility is that estradiol induces HSCs to undergo asymmetric self-renewal. In a recently described pathway, HSCs asymmetrically divide, yielding myeloid-restricted progenitors that retain long-term repopulating activity but are lineage-committed to megakaryocyte-erythroid cells (Yamamoto et al., 2013). This mechanism may support the increased demand for erythropoiesis during pregnancy.

Alternately, estradiol may act on preexisting myeloid-biased HSCs to increase their cell cycle entry and accelerate differentiation into MEPs. Higher levels of TGF $\beta$  have been implicated in regulation of HSC quiescence and inhibition of cycling of lymphoid-biased HSCs, while lower amounts were shown to promote cycling and myeloid gene expression programs in myeloid-biased HSCs (Challen et al., 2010). Notably, estradiol inhibits TGF $\beta$  signaling (Band and Laiho, 2011) and therefore may dampen TGF $\beta$  signaling to levels

necessary to drive myeloid-biased HSCs into cycle and allow subsequent MEP differentiation.

HSC subsets likely arise due to epigenetic modifications that instruct lineage-specific gene expression programs (Copley et al., 2012). Chronic exposure of HSCs to higher levels of estradiol in females may promote specific pathways of HSC specialization via ER-mediated epigenetic regulatory mechanisms. Nakada et al show that HSCs isolated from mice exposed to exogenous estradiol have enrichment of cell cycle genes and genes harboring a binding motif for the transcription factor E2F1, which is itself regulated by estradiol. Future experiments could determine if an estradiol-induced gene expression program is restricted to a subset of HSCs or promotes MEP differentiation.

A definitive property of HSCs is their ability to give rise to long-term self-renewing HSCs upon serial transplantation. Although Nakada et al did not demonstrate if estradiol alters this property of HSCs, an earlier report showed that relative to control mice, the LSK population in estradiol-treated mice is less quiescent and yields superior immune reconstitution upon transplant to irradiated mice (Illing et al., 2012). However, the estradiol-exposed HSCs may become exhausted sooner, since their serial transplant eventually leads to reduced numbers of granulocytes, although not lymphocytes.

Inflammatory signals including type I IFN and Toll-like receptor ligands also drive HSCs into cycle, but ultimately lead to HSC exhaustion, as well as promoting a myeloid bias of HSCs (Schuettpelez and Link, 2013). It would be fascinating to know if ER signaling in females accelerates the inflammation-induced HSC cycling, and if this contributes to the female bias in autoimmunity.

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## References

- Band AM, Laiho M. Crosstalk of TGF-beta and estrogen receptor signaling in breast cancer. *J Mammary Gland Biol Neoplasia*. 2011; 16:109–115. [PubMed: 21390570]
- Challen GA, Boles NC, Chambers SM, Goodell MA. Distinct hematopoietic stem cell subtypes are differentially regulated by TGF-beta1. *Cell Stem Cell*. 2010; 6:265–278. [PubMed: 20207229]
- Copley MR, Beer PA, Eaves CJ. Hematopoietic stem cell heterogeneity takes center stage. *Cell Stem Cell*. 2012; 10:690–697. [PubMed: 22704509]
- Illing A, Liu P, Ostermay S, Schilling A, de Haan G, Krust A, Amling M, Chambon P, Schinke T, Tuckermann JP. Estradiol increases hematopoietic stem and progenitor cells independent of its actions on bone. *Haematologica*. 2012; 97:1131–1135. [PubMed: 22371175]
- Kovats, S.; Carreras, E.; Agrawal, H. Sex steroid receptors in immune cells. In: Klein, SL.; Roberts, CW., editors. *Sex Hormones and Immunity to Infection*. Springer; 2010. p. 53-91.
- Medina KL, Garrett KP, Thompson LF, Rossi MI, Payne KJ, Kincade PW. Identification of very early lymphoid precursors in bone marrow and their regulation by estrogen. *Nat Immunol*. 2001; 2:718–724. [PubMed: 11477408]
- Nakada, et al.
- Notta F, Doulatov S, Dick JE. Engraftment of human hematopoietic stem cells is more efficient in female NOD/SCID/IL-2Rgc-null recipients. *Blood*. 2010; 115:3704–3707. [PubMed: 20207983]
- Schuettpelez LG, Link DC. Regulation of hematopoietic stem cell activity by inflammation. *Front Immunol*. 2013; 4:204. [PubMed: 23882270]

Yamamoto R, Morita Y, Ooehara J, Hamanaka S, Onodera M, Rudolph KL, Ema H, Nakauchi H. Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells. *Cell*. 2013; 154:1112–1126. [PubMed: 23993099]