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Author manuscript

*Cell Immunol.* Author manuscript; available in PMC 2016 April 01.

Published in final edited form as:

*Cell Immunol.* 2015 April ; 294(2): 63–69. doi:10.1016/j.cellimm.2015.01.018.

## Estrogen receptors regulate innate immune cells and signaling pathways

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### Abstract

Humans show strong sex differences in immunity to infection and autoimmunity, suggesting sex hormones modulate immune responses. Indeed, receptors for estrogens (ER) regulate cells and pathways in the innate and adaptive immune system, as well as immune cell development. ERs are ligand-dependent transcription factors that mediate long-range chromatin interactions and form complexes at gene regulatory elements, thus promoting epigenetic changes and transcription. ERs also participate in membrane-initiated steroid signaling to generate rapid responses. Estradiol and ER activity show profound dose- and context-dependent effects on innate immune signaling pathways and myeloid cell development. While estradiol most often promotes the production of type I interferon, innate pathways leading to pro-inflammatory cytokine production may be enhanced or dampened by ER activity. Regulation of innate immune cells and signaling by ERs may contribute to the reported sex differences in innate immune pathways. Here we review the recent literature and highlight several molecular mechanisms by which ERs regulate the development or functional responses of innate immune cells.

### Keywords

Estrogen; Estrogen Receptor; Sex Hormone; Dendritic Cells; Antigen-presenting cells; Macrophages; Monocytes; Cellular differentiation; Inflammation; Innate immune signaling; Hematopoiesis; Sex differences

## 1. ER expression in immune cells

ER  $\alpha$  and  $\beta$  proteins are members of the nuclear receptor super family encoded by the *ESR1* and *ESR2* genes, respectively [1]. Single ER chains form  $\alpha\alpha$ ,  $\beta\beta$  and  $\alpha\beta$  dimers, each of which is functionally distinct. As described below, ER-mediated mechanisms influence both the development and function of innate immune cells. Published studies document that ER mRNAs or proteins are expressed by hematopoietic progenitors and mature immune cells

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(see Table 1). Although ERs are regulated by transcriptional and post-transcriptional mechanisms, few studies have comprehensively determined relative ER RNA and protein levels in different immune cell types.

At least two studies have quantitatively assessed the relative *ESR1* and *ESR2* gene expression in human PBMC subsets (Table 1) [2, 3]. B cells express the highest levels of *ESR1* RNA, while CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, NK cells, and plasmacytoid DC express intermediate levels. Monocytes have the lowest levels of *ESR1* RNA, and interestingly, this is increased in monocyte-derived DCs, suggesting that *ESR1* is induced during DC differentiation. *ESR2* RNA is expressed at the highest levels in B cells and plasmacytoid DCs, and at low levels in other cell types. Human monocytes and monocyte-derived DCs, and blood myeloid and plasmacytoid DCs alter their functional responses upon exposure to estrogens [4–6].

Mature immune cells in murine lymphoid organs express *Esr1* (encoding ER $\alpha$ ), and in some cases *Esr2* (encoding ER $\beta$ ). Murine lymphocytes (B, T and NK cells) contain *Esr1* and ER $\alpha$  [7–10], and B and NK cells were reported to express ER $\beta$  protein [7, 8]. Murine splenic DCs (including conventional and plasmacytoid DCs), as well as bone marrow-derived DCs, express *Esr1* and ER $\alpha$  but negligible *Esr2* and ER $\beta$  [6, 9, 11, 12]. Bone marrow-derived and peritoneal macrophages also express *Esr1* but little if any *Esr2* [9, 13]. However, some populations of DCs *in vivo*, such as DCs infiltrating the central nervous system during experimental autoimmune encephalomyelitis (EAE), do express ER $\beta$  [14], suggesting that ER $\beta$  may be induced in activated myeloid cells.

While sex differences in ER expression in the brain are well documented [15, 16], only a few publications report sex differences in ER RNA or protein expression in primary immune cells. Monocytes in premenopausal women contain lower amounts of *ESR1* RNA than monocytes isolated from males and postmenopausal women, suggesting that higher estradiol levels correlate with reduced *ESR1* expression [3]. In contrast, *ESR1* and *ESR2* RNA levels did not differ in male and female B and T lymphocytes, or in lymphocytes of pre- and postmenopausal women [3]. Human plasmacytoid DCs in females and males also did not differ in levels of *ESR1* and *ESR2* RNA [2].

The molecular mechanisms leading to sex differences in ER expression in particular immune cells are yet to be defined. ER RNA and protein levels are autoregulated [17]. However, mechanisms by which different concentrations of estrogens in males and females may lead to sex differences in ER expression in some cell types, but not others, remain unclear, but are likely due to epigenetic regulatory pathways.

Hematopoietic progenitors in human and murine bone marrow also express ERs. CD34<sup>+</sup> hematopoietic stem cells (HSCs) in human adult bone marrow, but not cord blood, express both *ESR1* and *ESR2* [18]. In mice, *Esr1* is expressed by adult bone marrow hematopoietic progenitors [defined as lineage-negative Sca-1<sup>+</sup> c-kit<sup>+</sup> (LSK)], but not by fetal liver progenitors [18, 19]. A recent study with highly purified murine HSCs (defined as LSK CD150<sup>+</sup> CD48<sup>-</sup>) showed that female and male HSCs express *Esr1* but not *Esr2* [20]. This study also showed that female HSCs contain lower amounts of *Esr1* RNA than male HSCs

[20]. Murine myeloid progenitors (lineage-negative Sca-1<sup>-</sup> c-kit<sup>+</sup> Flt3<sup>+</sup>) express *Esr1* but not *Esr2* [19]. Of note, data reported in the Immunological Genome Project ([www.immgen.org](http://www.immgen.org)) show that murine hematopoietic progenitors including HSCs, the CLP (common lymphoid progenitor), the ETP (early T lineage progenitor) and myeloid cell progenitors contain significantly more *Esr1* RNA than mature immune cells.

In addition to full-length ER $\alpha$  and ER $\beta$  proteins, splice variants leading to truncated proteins have been described. For example, human macrophages predominantly express the N-terminal truncated ER $\alpha$ 46 protein, which is regulated by estradiol [21].

## 2. ER signaling mechanisms

ERs are ligand-dependent transcription factors that mediate long-range chromatin interactions. ERs form complexes at specific DNA sites with chromatin-modifying coregulators and other transcription factors, leading to epigenetic modifications of chromatin as well as transcription initiation [22]. The nuclear or “genomic” actions of ERs mediate many physiological effects of estrogens.

Studies of breast cancer cells have revealed mechanisms for the recruitment and action of ERs at specific sites on DNA [23]. Lineage-specific epigenetic marking (via post-translational modification of histones) of DNA regulatory elements guides pioneer factors that remodel compacted chromatin, increasing accessibility of specific DNA sites to transcription factors such as ER $\alpha$ . The pioneer factors found at >80% of ER $\alpha$  binding sites include FOXA1, PBX1, AP-2 $\gamma$ , TLE1, PBX1 and GATA3. ER $\alpha$  is preferentially recruited to DNA elements present in open chromatin, characterized by DNase I hypersensitivity sites. ER $\alpha$  binds to regulatory elements or enhancers that are distant (e.g. 10–100 kb) from the promoters of target genes, and is involved in establishing chromatin loops that bridge these distal ER $\alpha$  bound elements to transcription start sites [24]. Once bound, ER $\alpha$  forms complexes with coactivator proteins that carry out chromatin modification, leading to increased recruitment of RNA polymerase II and additional chromatin remodeling. The activation function AF-1 and AF-2 domains mediate the DNA binding activity of ERs. Mutants of these domains have been used to determine if a particular estrogen response requires the DNA binding activity of ER.

Nuclear ERs directly bind estrogen response elements (EREs), or are tethered indirectly to DNA by forming complexes with other transcription factors that bind DNA via their own consensus sequences [25]. ERs often bind to DNA associated with transcription factors (e.g. NF- $\kappa$ B, SP1, AP-1, C/EBP $\beta$ ) that are important for immune cell function [26]. Structurally distinct ligands impart specific unique conformations to ER dimers, and this regulates the recruitment of coregulators and histone-modifying enzymes into multi-protein transcription complexes [1]. Ligand-free ER $\alpha$  also can participate in transcriptional complexes [27]. The complex of ER $\alpha$ , coregulators and histone-modifying enzymes leads to post-translational histone modifications (acetylation, phosphorylation, methylation) that alter chromatin structure [22]. Transcriptional coregulators act as coactivators or corepressors (or both), and remodel chromatin in configurations that are permissive or inhibitory for transcription. ERs can recruit coactivators such as SRC1 that have histone acetyltransferase activity, or they

can interact with acetyltransferases such as p300/CBP, leading to transcriptional activation. ER corepressors such as NCOR can complex with histone deacetylases, leading to gene repression.

Estrogens also elicit rapid (seconds to minutes) nonnuclear (“nongenomic”) signal transduction, now termed membrane-initiated steroid signaling (MISS). The rapid MISS effects include mobilization of intracellular calcium, generation of cAMP, modulation of potassium currents, phospholipase C activation, nitric oxide production and the stimulation of protein kinase pathways such as PI3K/AKT and ERK [28]. MISS is mediated by a fraction of ER $\alpha$  that is associated via a palmitoylated site (Cys 447 in humans) with the cytosolic face of the plasma membrane. In the plasma membrane, ER $\alpha$  is localized to caveolae/lipid rafts by direct binding to caveolin-1 through Ser 522 or indirectly via scaffold proteins [28]. MISS responses have been studied using synthetic estrogen-macromolecule conjugates that cannot enter the nucleus [29], or by mutating the palmitoylation site at Cys 447 (Cys 451 in mice) [30, 31].

Active research is addressing whether membrane-initiated and transcriptional activities of ERs are integrated to produce one unique effect or represent parallel pathways with distinct effects. The two pathways can be distinguished using human or murine loss of function mutants, which abolish either the AF-2 domain required for the nuclear pathway, or eliminate membrane localization via mutation of the C447 palmitoylation site. In murine models, this approach showed that some estradiol-induced events require only one of the two mechanisms, and this depends on the target tissue. AF-2 mutant mice selectively lack nuclear ER actions, while mice lacking the C451 palmitoylation site are selectively deficient in known membrane ER actions [30]. Analyses of gene expression in the uterus showed that the majority of estradiol-dependent gene regulation is dependent on the AF-2 domain rather than membrane localization, suggesting minimal crosstalk between the two pathways. However, a study with human cells showed that the membrane ER localization via palmitoylation is important for delaying ER $\alpha$  degradation, and is required for ER $\alpha$  S118 phosphorylation and estradiol-induced ER $\alpha$  occupancy at ERE sites [31]. These data suggest that ER $\alpha$  membrane localization and extranuclear signaling can be a prerequisite for ER $\alpha$  transcriptional activity. This is consistent with prior work showing that MISS pathways can lead to histone modifications and altered chromatin structure near estrogen-regulated genes [1, 22].

Investigation of mechanisms by which ligand-bound ERs modulate gene expression programs in immune cells or their precursors will increase our understanding of how estrogens modulate immunity. While recent studies of AF-1- or AF-2-deficient mice, and mice bearing an ER $\alpha$  mutant that cannot bind ERE, have advanced this field [32, 33], we still lack a detailed understanding of the mechanisms of ER-mediated nuclear or MISS responses in innate immunity.

### 3. ERs regulate innate immune signaling pathways

ER activity has been shown to augment *and* dampen innate immune signaling pathways in dendritic cells and macrophages. An emerging theme is that ER $\alpha$  and physiological adult

levels of estradiol promote the production of type I interferon (IFN). However, estradiol and ERs have been reported to exert either positive or negative regulatory effects on pro-inflammatory cytokine production; this varies with the cell type or estrogen dose. Pregnancy or higher doses of ectopic estrogens typically suppress immune responses. Sex differences in expression of genes involved in innate immunity also have been reported, and in some cases these differences are abolished in gonadectomized mice or differ in pre- and postmenopausal women, suggesting that ER signaling may regulate these genes [34, 35].

ER activity may regulate the function of innate immune cells via one of several mechanisms outlined here, or by additional mechanisms such as induction of microRNAs [36, 37].

(i) ER-induced epigenetic changes in DC precursors may alter gene expression programs governing mature DC functional responses. For example, due to increased levels of circulating estradiol, DCs that develop in adult female mice may harbor more open chromatin at genes encoding innate immune signaling intermediates and type I IFN than DCs in male mice. This might explain the more robust production of type I IFN observed in females. In the case of pregnancy, exposure of differentiating DCs to the high estradiol levels could result in epigenetic changes in a gene program that promotes a tolerogenic DC phenotype.

(ii) ERs may act acutely during mature DC activation to directly regulate gene expression. For example, upon DC stimulation via pattern recognition receptors or cytokines, ERs may be recruited directly to DNA regulatory elements to promote or inhibit expression of genes involved in innate immunity. As described below, ER $\alpha$  forms complexes with or regulates multiple NF- $\kappa$ B pathway members to control cytokine responses. Furthermore, ER $\alpha$  may have distinct molecular effects when it is ligand-bound vs. ligand-free, and this will be influenced by ligand concentration and structure.

(iii) ER expression itself may change when innate cells are stimulated. For example, LPS stimulation leads to a decrease in *Esr1* RNA in endothelial cells [38]. If ER $\alpha$  were to normally participate in an inhibitory complex to repress a gene, a decrease in ER $\alpha$  expression after Toll-like receptor (TLR) signaling might allow increased expression of pro-inflammatory genes. In contrast, IFN $\alpha$  stimulation of murine splenocytes leads to increased levels of both *Esr1* RNA and ER $\alpha$  protein [39]. This leads to increased expression of both IFN $\alpha$  and ER responsive genes.

It is also of interest to consider how differences in ER expression levels or in ER ligand concentration could lead to differential ER signaling in females and males. Although examples of gene expression differences in males and females have been reported, few studies have directly correlated these transcriptional profiles with ER RNA or protein expression or with an independent measure of the strength of ER signaling. Such a study might be additionally complicated by the dynamic transcriptional and post-transcriptional regulation of ER levels that occurs upon cellular activation. An *in vivo* reporter of ER activity might help to correlate ER expression with the strength of ER signaling, and to determine if this differs in females and males [40].

### 3.1. Induction of Type I Interferon

Multiple reports show that estradiol acting via ER $\alpha$  promotes the production of type I IFN. This may be due to induction of genes in the innate stimuli sensing pathways or direct regulation of type I IFN genes. Indeed, a body of work shows that estradiol promotes type I IFN-inducible innate pathways in murine splenocytes, including expression of the *Unc93b1*, *p202*, *Aim2*, *Irf5*, and *Baff* genes [41–43]. Consistent with this, these studies also show that these IFN pathway genes are expressed at higher levels in females at baseline and after IFN stimulation [41–43]. These studies support the hypothesis that ER $\alpha$  and interferons interact in a mutually positive feedback loop to regulate innate immunity [39]. TRIM-21 also is induced by estradiol/ER $\alpha$  in human monocytes, and this leads to increased IRF3 stability and production of IFN $\beta$  and IL-23 [44]. Estradiol also increases expression of the *TLR8* gene in human PBMCs [45].

Models of mice bearing conditional alleles of *Esr1* and specific promoter-driven Cre recombinase enable the study of cell type-specific ER deficiency in the context of physiologically normal cycling levels of ER ligands. Mice lacking ER $\alpha$  in CD11c<sup>+</sup> DC were used to study DC responses to TLR ligands [6]. Upon injection of nucleic acid ligands of TLR7 and TLR9, ER $\alpha$ -deficient pDCs in females with intact ovaries produced less IFN $\alpha$ , showing that ER $\alpha$  signaling acts directly in pDCs to promote IFN $\alpha$  synthesis. This type of study will help to identify sex differences in ER-regulated immune responses in specific cell types and in response to distinct estradiol levels *in vivo*.

Human female PBMCs or pDCs also produce significantly more IFN $\alpha$  in response to viral nucleic acids or synthetic TLR7 ligands than male cells [46, 47]. The sex difference could not be reproduced by short-term *in vitro* exposure to estradiol, suggesting that long-term exposure to higher levels of estrogens in pre-menopausal females leads to greater pDC function. In support of this, a one-month treatment of post-menopausal women with estradiol leads to a greater fraction of blood pDC capable of producing IFN $\alpha$  upon stimulation via TLR7 or TLR9 [6]. These data suggest that the action of estradiol during DC differentiation increases pDC functional capacity. Indeed, transfer of human female CD34<sup>+</sup> hematopoietic stem cells into female or male immunodeficient mice showed that the human pDCs that develop in female mice have a greater capacity to produce IFN $\alpha$  [6]. A second study in which human female or male CD34<sup>+</sup> stem cells were transferred into male or female recipient mice showed that the sex of the donor cells also influences type I IFN production, with female pDCs having a greater intrinsic capacity to produce IFN $\alpha$  even in a male host [2]. This result could be due to XX chromosome content in female cells, as incomplete X inactivation could lead to increased expression of X-linked genes. Alternately, the female CD34<sup>+</sup> cells may bear distinct epigenetic marks acquired during their own development that could influence the function of descendent pDCs. These studies help to explain differences in pDC function that occur between males and females, as well as in pre- and post-menopausal women.

The ability of estradiol and ER signaling to augment these type I Interferon pathways may help to explain the profound female sex bias in systemic lupus erythematosus, an autoimmune disease characterized by high levels of IFN [48]. The crosstalk of the ER and



type I IFN pathways also may help to explain sex differences in pathogenesis of HIV, as well as the ability of estradiol to protect HIV-infected cells. As mentioned above, pDCs from female HIV patients produce more IFN $\alpha$  than male pDCs [46]. Estradiol also induces type I IFN in human monocyte-derived macrophages, thus protecting them from HIV infection [49]. A second study showed that estradiol reduces the susceptibility of macrophages to HIV infection, possibly by inhibiting virus entry, which may be related to the ability of estradiol to increase expression of IFN-induced antiviral genes [50].

### 3.2 Pro-inflammatory cytokine responses

Multiple studies show that ER $\alpha$  promotes production of proinflammatory cytokines in response to TLR ligand stimulation of dendritic cells and macrophages. pDCs isolated from ER $\alpha^{-/-}$  lupus prone mice and stimulated with TLR9 ligands produce less IL-6 [51]. Other work showed that ER $\alpha^{-/-}$  bone marrow derived DCs stimulated with TLR ligands produce lower amounts of IL-6, IL-23, IL-12 and IL-1 $\beta$ , in some cases due to decreased transcription of cytokine genes [32, 51–53]. This work is consistent with studies showing that estradiol increases production of IL-12 by DCs [52, 54]. Deletion of a conditional ER $\alpha$  allele preferentially in myeloid cells using LysM-driven Cre showed that estradiol enhances TLR4 signaling in macrophages *in vivo*, leading to increased IL-6, IL-1 $\beta$  and iNOS [55]. Estradiol augments this pathway by reducing inhibitory PI3K signaling and Akt phosphorylation in macrophages. These and similar studies help to explain the superior immune response to infection often observed in females [56].

In contrast, work in several disease models has shown that levels of systemic estrogens at the higher end of the normal spectrum promote anti-inflammatory responses. Estrus levels of estradiol reduce the ability of murine DCs to secrete IL-23 and promote T<sub>H</sub>17 responses during *Candida albicans* infection [57]. Female mice infected with influenza experience greater morbidity and mortality than males, which correlates with higher induction of proinflammatory cytokines and chemokines such as TNF $\alpha$ , IFN $\gamma$ , IL-6 and CCL2 in females [58]. In this model, administration of high doses of estradiol protects females against damaging excessive inflammatory responses in the lung through recruitment of neutrophils; this ER $\alpha$ -mediated response leads to increased TNF $\alpha$  and IFN $\gamma$  production by virus-specific CD8<sup>+</sup> T cells in the lungs [58, 59].

The high levels of estradiol and estriol in pregnancy also decrease pro-inflammatory immune responses. Mice treated with estriol contain tolerogenic DCs expressing increased levels of inhibitory costimulatory molecules such as PD-L1 and immunoregulatory cytokine mRNAs including *Ill10* and *Tgfb*. Adoptive transfer of these estriol treated DCs protects against inflammatory autoimmune EAE [60]. Human DCs treated with pregnancy levels of estradiol show a decreased antiviral response to RNA viruses due to suppression of type I IFN synthesis and capacity to stimulate naive CD4 T cells [5, 61].

The mechanisms by which low and high physiological doses of estrogens differentially alter ER activity to influence expression of immune response genes are unclear. One possibility is that low and high levels of estradiol induce distinct ER-containing transcriptional complexes, and this leads to different patterns of epigenetic marks on genes within functional pathways that promote or dampen inflammation. As described below, regulation

of the NF- $\kappa$ B pathway by estrogens and ERs is complex, and more work is needed to fully understand the ability of ER signaling to both augment and inhibit cytokines dependent on NF- $\kappa$ B or other innate signaling pathways.

### 3.3 Inhibition of NF- $\kappa$ B signaling

ER $\alpha$  participates in NF- $\kappa$ B transcriptional complexes, in many cases with inhibitory effects on both ER and NF- $\kappa$ B mediated transcriptional activity [62]. The inhibition of the NF- $\kappa$ B pathway by ERs often limits the extent of the inflammatory response and occurs by several mechanisms [63]. Some of these molecular mechanisms are highlighted here, as they provide instructive examples of how ERs can regulate gene expression. ERs also have been reported to have regulatory interactions with many other transcription factors (e.g. SP1, AP-1) involved in innate immune pathways.

(i) *Displacement of coactivators.* ER $\alpha$  and NF- $\kappa$ B p65 interact directly on the promoter of the *MCPI* gene, but ER $\alpha$  displaces the coactivator CBP (cAMP response element-binding protein (CREB)-binding protein), leading to suppression of the gene [64]. In a similar mechanism, ER displaces p65 and associated coregulators from the IL-6 gene [64]. (ii) *Recruitment of corepressors.* TNF $\alpha$  stimulation induces a complex of NF- $\kappa$ B p50-p65, CBP and ligand-free ER that leads to transcription of the *TNFA* gene. However, when estradiol is present and the complex contains ligand-bound ER, the corepressor GRIP1 is recruited to the complex and the *TNFA* gene is suppressed [27]. Similarly, the HPV16 E7 protein induces a transcriptional repressor complex involving ER $\alpha$  on the *TLR9* promoter in human epithelial cells [65]. An inhibitory complex of NF- $\kappa$ B p50-p65 plus ER $\alpha$  (phosphorylated at Ser 118) recruits the histone demethylase JARID1b and histone deacetylase HDAC1, leading to decreased methylation and acetylation of histones upstream of the *TLR9* transcription start site. An ERE is within 200bp of the NF- $\kappa$ B cis element. This inhibitory ER $\alpha$ -NF- $\kappa$ B complex suppresses TLR9 expression, thus decreasing production of type I IFN (and IFN-induced antiviral genes) and pro-inflammatory cytokines. (iii) *Enhancing I $\kappa$ B $\alpha$  levels.* Estradiol promotes the new synthesis of I $\kappa$ B $\alpha$ , thus inhibiting NF- $\kappa$ B signaling and inflammatory gene expression in smooth muscle cells [66]. (iv) *Inhibition of NF- $\kappa$ B nuclear translocation.* Estradiol inhibits the nuclear translocation of p65, c-Rel and RelB. Despite this, estradiol increases cytokine production in murine splenocytes by inducing the association of Bcl-3 and NF- $\kappa$ B p50 and promoting the posttranslational modification of Stat1 and NF- $\kappa$ B subunits [67, 68]. (v) *Repression of I $\kappa$ B $\kappa$ G.* I $\kappa$ B $\kappa$ G is the regulatory subunit of the inhibitor of NF- $\kappa$ B kinase (IKK) complex, which degrades I $\kappa$ B $\alpha$ , leaving NF- $\kappa$ B dimers free to translocate into the nucleus. Estrus levels of estradiol decrease *I $\kappa$ B $\kappa$ G* transcription in murine dendritic cells. This limits IKK activity and reduces the nuclear translocation of NF- $\kappa$ B [69].

## 4. Regulation of immune cell differentiation by estrogen receptors

As described below, ER $\alpha$  acts directly in HSCs, lymphoid progenitors and myeloid progenitors to promote developmental pathways. ER action in hematopoietic progenitors may have several consequences: (i) ERs may induce epigenetic changes in precursors that influence downstream developmental pathways or functional responses in mature cells. (ii)



ERs may directly promote a developmental pathway by binding directly to a specific gene or genes within a pathway.

Multiple studies have shown that ER signaling regulates hematopoietic progenitor populations during homeostasis, and this likely regulates the number and type of immune cells present *in vivo*. However, because estradiol and ERs have been implicated in the inflammation that occurs during infection and autoimmunity, it is important to consider the effects of ER signaling on new immune cell development during inflammation. The development of innate immune cells may be particularly susceptible to changes in ER ligand concentration in the host due to their short lifespan (days). Augmentation of the inflammatory pathway of DC development by ER $\alpha$  signaling may lead to increased numbers of inflammatory DCs in tissues and lymphoid organs that can direct innate and adaptive immune responses. Examples of ER signaling in HSCs and in myeloid progenitors are described below.

#### 4.1. Hematopoietic stem cells

A recent study shows that HSCs divide more frequently in females than in males [20]. The increased HSC cycling requires HSC-intrinsic ER $\alpha$  and is driven by endogenous estradiol in females: both ovariectomy and an aromatase inhibitor reduce cycling to levels seen in males. Pregnancy also induces a significant increase in HSC division. Interestingly, the lower levels of estradiol in unmanipulated males are insufficient to promote increased HSC cycling, suggesting threshold amounts of estradiol are necessary for HSC self-renewal. Although androgens modulate lymphopoiesis [70, 71], HSCs do not express androgen receptors and their cycling is not driven by testosterone.

Despite their increased self-renewal, HSC numbers do not differ in females and males. However, 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 [20]. HSC subsets with distinct lineage-specific gene expression programs likely arise due to epigenetic modification [72], and higher levels of estradiol may promote the specialization to MEP. HSCs isolated from mice exposed to exogenous estradiol have enrichment of cell cycle genes and genes bearing a binding motif for the transcription factor E2F1 [20], which is itself regulated by estradiol [73]. These data suggest that an estradiol-induced gene expression program may be restricted to a subset of HSCs or promote MEP differentiation.

Thus, ERs may respond to higher estrogen levels in females to generate female-specific epigenetic modifications of chromatin in HSCs. This ER effect could modulate the HSCs directly, or it could alter the responses of descendent precursors and mature immune cells. Effects of estradiol/ER signaling in hematopoietic progenitor engraftment have been reported. The LSK population in estradiol-treated mice is less quiescent and yields superior immune reconstitution upon transplant to irradiated mice [74]. Similarly, transplantation of human HSCs into immunodeficient mice is superior when the recipients are female [75]. Interestingly, HSCs isolated from autoimmune female, but not male, mice show intrinsic capacity to induce lupuslike autoimmunity in both female and male recipients [76]. This is consistent with long-term epigenetic effects of ER signaling in the donor female HSCs.

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 [77]. Interestingly, HSCs in estradiol-treated mice appear to become exhausted sooner, since their serial transplant eventually leads to reduced numbers of granulocytes, although not lymphocytes [74]. It is possible that ER signaling in females accelerates inflammation-induced HSC cycling, thus contributing to the female bias in autoimmunity.

#### 4.2. Dendritic cell development

Experiments with mice pregnant or treated with supra-physiological estradiol levels showed that variations in systemic estrogen levels *in vivo* regulate development of plasmacytoid DC (pDC) and B lymphocytes via effects on the common lymphoid progenitor (CLP) and lymphoid-primed multipotent progenitors [78–81]. In addition, estradiol acts via ER $\alpha$  in myeloid progenitors to decrease Flt3L-driven DC differentiation, a pathway predominant in homeostasis [19]. These data suggest that estradiol/ER signaling regulates hematopoietic progenitor homeostasis to limit the number of myeloid and lymphoid progenitors in the steady state.

In contrast, we and others have determined that estradiol and ER $\alpha$  signaling promote the GM-CSF-mediated differentiation of DCs from murine hematopoietic progenitors or human and rat monocytes [11, 12, 19, 52, 53, 82–84]. Estradiol acts in highly purified ER $\alpha$ <sup>+</sup> ER $\beta$ <sup>-</sup> myeloid progenitors (Lin<sup>-</sup> c-kit<sup>+</sup> Sca-1<sup>-</sup> Flt3<sup>+</sup> IL-7R $\alpha$ <sup>-</sup>) in murine bone marrow to promote GM-CSF-mediated DC differentiation [19]. Kinetic experiments showed that estradiol signals via ER $\alpha$  to promote differentiation of myeloid progenitors within the first 24 hours of culture, suggesting an effect on initiation of DC differentiation [82]. At the molecular level, ER $\alpha$  signaling increases levels of interferon regulatory factor 4 (IRF4), a transcription factor that acts downstream of the GM-CSF receptor to promote DC differentiation [32, 82]. These studies were recently reviewed [85].

The results in the GM-CSF-driven culture model suggest that ER $\alpha$  signaling promotes new DC differentiation during inflammation *in vivo*. To determine the relative efficiency of DC differentiation from ER $\alpha$ <sup>+</sup> and ER $\alpha$ <sup>-/-</sup> bone marrow precursors *in vivo*, we analyzed the CD11c<sup>+</sup> CD11b<sup>+</sup> DC subset present in mixed ER $\alpha$ <sup>+</sup>/ER $\alpha$ <sup>-/-</sup> bone marrow chimeric mice at an early time point (3 weeks) post-reconstitution when myeloid cells have developed. In this model, DC development occurs in the post-radiation inflammatory environment in which both GM-CSF and Flt3L may be elevated. In the chimeric mice, newly differentiated CD11b<sup>+</sup> DCs in bone marrow are derived preferentially from ER $\alpha$ <sup>+</sup> donor cells (~2:1 ratio of ER $\alpha$ <sup>+</sup> to ER $\alpha$ <sup>-/-</sup> DCs) [82]. These data show that during radiation-induced inflammation and hematopoiesis *in vivo*, ER $\alpha$  signaling promotes CD11b<sup>+</sup> DC development from short term repopulating bone marrow progenitors.

## 5. Conclusion

A significant body of work now shows that estradiol and ER signaling regulate inflammatory pathways of innate immune cells, including dendritic cells and macrophages. Lower physiological levels of estradiol generally promote pathways leading to production of type I IFN, and often pro-inflammatory cytokines. However, in some cases ER signaling

dampens these pathways even in lower estrogen environments. Higher physiological or supraphysiological levels of estrogens most often foster anti-inflammatory responses that attenuate inflammation. Similarly, ER signaling promotes or dampens new immune cell development through effects on hematopoietic progenitors. Despite these advances, we still lack detailed information about the molecular mechanisms of ER signaling in immune cells. The majority of studies have focused on the nuclear activity of ligand-bound ERs, with an emphasis on specific gene regulation. However, new tools to study membrane-initiated ER signaling may help to understand more rapid responses to estradiol in innate immune cells. Genome-wide analyses of epigenetic marks or ER binding sites in response to estradiol/ER signaling in innate immune cells would provide a more comprehensive picture of regulatory mechanisms. This type of work would reveal whether ERs act to mediate epigenetic changes at multiple genes linked in a pathway, or alternately, if ERs regulate specific pathways by acting to promote or inhibit a master regulator. Ultimately, investigation of ER-mediated regulatory mechanisms with new available molecular tools will increase our understanding of how estrogens and sex differences lead to profound effects on immunity and autoimmunity.

## Acknowledgements

S.K. was supported by NIH grants AI092511, AI083715 and HL119501.

## References

1. Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, Tujague M, Strom A, Treuter E, Warner M, Gustafsson JA. Estrogen receptors: how do they signal and what are their targets. *Physiol Rev.* 2007; 87:905–931. [PubMed: 17615392]
2. Laffont S, Rouquie N, Azar P, Seillet C, Plumas J, Asford C, Guery JC. X-Chromosome Complement and Estrogen Receptor Signaling Independently Contribute to the Enhanced TLR7-Mediated IFN- $\alpha$  Production of Plasmacytoid Dendritic Cells from Women. *J Immunol.* 2014; 193:5444–5452. [PubMed: 25339659]
3. Phiel KL, Henderson RA, Adelman SJ, Elloso MM. Differential estrogen receptor gene expression in human peripheral blood mononuclear cell populations. *Immunol Lett.* 2005; 97:107–113. [PubMed: 15626482]
4. Mor G, Sapi E, Abrahams VM, Rutherford T, Song J, Hao XY, Muzaffar S, Kohen F. Interaction of the estrogen receptors with the Fas ligand promoter in human monocytes. *J Immunol.* 2003; 170:114–122. [PubMed: 12496390]
5. Escribese MM, Kraus T, Rhee E, Fernandez-Sesma A, Lopez CB, Moran TM. Estrogen inhibits dendritic cell maturation to RNA viruses. *Blood.* 2008; 112:4574–4584. [PubMed: 18802009]
6. Seillet C, Laffont S, Tremollieres F, Rouquie N, Ribot C, Arnal JF, Douin-Echinard V, Gourdy P, Guery JC. The TLR-mediated response of plasmacytoid dendritic cells is positively regulated by estradiol in vivo through cell-intrinsic estrogen receptor alpha signaling. *Blood.* 2012; 119:454–464. [PubMed: 22096248]
7. Grimaldi CM, Cleary J, Dagtas AS, Moussai D, Diamond B. Estrogen alters thresholds for B cell apoptosis and activation. *J Clin Invest.* 2002; 109 1625-133.
8. Curran EM, Berghaus LJ, Verneti NJ, Saporita AJ, Lubahn DB, Estes DM. Natural killer cells express estrogen receptor-alpha and estrogen receptor-beta and can respond to estrogen via a non-estrogen receptor-alpha-mediated pathway. *Cell Immunol.* 2001; 214:12–20. [PubMed: 11902825]
9. Lambert KC, Curran EM, Judy BM, Lubahn DB, Estes DM. Estrogen receptor-alpha deficiency promotes increased TNF-alpha secretion and bacterial killing by murine macrophages in response to microbial stimuli in vitro. *J Leukoc Biol.* 2004; 75:1166–1172. [PubMed: 15020652]

10. Lelu K, Laffont S, Delpy L, Paulet PE, Perinat T, Tschanz SA, Pelletier L, Engelhardt B, Guery JC. Estrogen receptor alpha signaling in T lymphocytes is required for estradiol-mediated inhibition of Th1 and Th17 cell differentiation and protection against experimental autoimmune encephalomyelitis. *J Immunol.* 2011; 187:2386–2393. [PubMed: 21810607]
11. Paharkova-Vatchkova V, Maldonado R, Kovats S. Estrogen preferentially promotes the differentiation of CD11c+ CD11b(intermediate) dendritic cells from bone marrow precursors. *J Immunol.* 2004; 172:1426–1436. [PubMed: 14734718]
12. Mao A, Paharkova-Vatchkova V, Hardy J, Miller MM, Kovats S. Estrogen selectively promotes the differentiation of dendritic cells with characteristics of Langerhans cells. *J Immunol.* 2005; 175:5146–5151. [PubMed: 16210618]
13. Ribas V, Drew BG, Le JA, Soleymani T, Daraei P, Sitz D, Mohammad L, Henstridge DC, Febbraio MA, Hewitt SC, Korach KS, Bensinger SJ, Hevener AL. Myeloid-specific estrogen receptor alpha deficiency impairs metabolic homeostasis and accelerates atherosclerotic lesion development. *Proc Natl Acad Sci U S A.* 2011; 108:16457–16462. [PubMed: 21900603]
14. Du S, Sandoval F, Trinh P, Umeda E, Voskuhl R. Estrogen receptor-beta ligand treatment modulates dendritic cells in the target organ during autoimmune demyelinating disease. *Eur J Immunol.* 2011; 41:140–150. [PubMed: 21182085]
15. Wilson ME, Westberry JM, Trout AL. Estrogen receptor-alpha gene expression in the cortex: sex differences during development and in adulthood. *Horm Behav.* 2011; 59:353–357. [PubMed: 20713055]
16. Zuloaga DG, Zuloaga KL, Hinds LR, Carbone DL, Handa RJ. Estrogen receptor beta expression in the mouse forebrain: age and sex differences. *J Comp Neurol.* 2014; 522:358–371. [PubMed: 23818057]
17. Castles CG, Oesterreich S, Hansen R, Fuqua SA. Auto-regulation of the estrogen receptor promoter. *J Steroid Biochem Mol Biol.* 1997; 62:155–163. [PubMed: 9393950]
18. Igarashi H, Kouro T, Yokota T, Comp PC, Kincade PW. Age and stage dependency of estrogen receptor expression by lymphocyte precursors. *Proc Natl Acad Sci U S A.* 2001; 98 15131-15136.
19. Carreras E, Turner S, Paharkova-Vatchkova V, Mao A, Dascher C, Kovats S. Estradiol Acts Directly on Bone Marrow Myeloid Progenitors to Differentially Regulate GM-CSF or Flt3 Ligand-Mediated Dendritic Cell Differentiation. *J Immunol.* 2008; 180:727–738. [PubMed: 18178810]
20. Nakada D, Oguro H, Levi BP, Ryan N, Kitano A, Saitoh Y, Takeichi M, Wendt GR, Morrison SJ. Oestrogen increases haematopoietic stem-cell self-renewal in females and during pregnancy. *Nature.* 2014; 505:555–558. [PubMed: 24451543]
21. Murphy AJ, Guyre PM, Wira CR, Pioli PA. Estradiol regulates expression of estrogen receptor ERalpha46 in human macrophages. *PLoS One.* 2009; 4:e5539. [PubMed: 19440537]
22. Mann M, Cortez V, Vadlamudi RK. Epigenetics of Estrogen Receptor Signaling: Role in Hormonal Cancer Progression and Therapy. *Cancers (Basel).* 2011; 3:1691–1707. [PubMed: 21814622]
23. Magnani L, Lupien M. Chromatin and epigenetic determinants of estrogen receptor alpha (ESR1) signaling. *Mol Cell Endocrinol.* 2014; 382:633–641. [PubMed: 23684889]
24. Liu MH, Cheung E. Estrogen receptor-mediated long-range chromatin interactions and transcription in breast cancer. *Mol Cell Endocrinol.* 2014; 382:624–632. [PubMed: 24071518]
25. O’Lone R, Frith MC, Karlsson EK, Hansen U. Genomic targets of nuclear estrogen receptors. *Mol Endocrinol.* 2004; 18:1859–1875. [PubMed: 15031323]
26. Leitman DC, Paruthiyil S, Vivar OI, Saunier EF, Herber CB, Cohen I, Tagliaferri M, Speed TP. Regulation of specific target genes and biological responses by estrogen receptor subtype agonists. *Curr Opin Pharmacol.* 2010; 10:629–636. [PubMed: 20951642]
27. Cvorova A, Tzagarakis-Foster C, Tatomer D, Paruthiyil S, Fox MS, Leitman DC. Distinct roles of unliganded and liganded estrogen receptors in transcriptional repression. *Mol Cell.* 2006; 21:555–564. [PubMed: 16483936]
28. Ueda K, Karas RH. Emerging evidence of the importance of rapid, non-nuclear estrogen receptor signaling in the cardiovascular system. *Steroids.* 2013; 78:589–596. [PubMed: 23276634]

29. Harrington WR, Kim SH, Funk CC, Madak-Erdogan Z, Schiff R, Katzenellenbogen JA, Katzenellenbogen BS. Estrogen dendrimer conjugates that preferentially activate extranuclear, nongenomic versus genomic pathways of estrogen action. *Mol Endocrinol*. 2006; 20:491–502. [PubMed: 16306086]
30. Adlanmerini M, Solinhac R, Abot A, Fabre A, Raymond-Letron I, Guihot AL, Boudou F, Sautier L, Vessieres E, Kim SH, Liere P, Fontaine C, Krust A, Chambon P, Katzenellenbogen JA, Gourdy P, Shaul PW, Henrion D, Arnal JF, Lenfant F. Mutation of the palmitoylation site of estrogen receptor alpha in vivo reveals tissue-specific roles for membrane versus nuclear actions. *Proc Natl Acad Sci U S A*. 2014; 111:E283–E290. [PubMed: 24371309]
31. La Rosa P, Pesiri V, Leclercq G, Marino M, Acconcia F. Palmitoylation regulates 17beta-estradiol-induced estrogen receptor-alpha degradation and transcriptional activity. *Mol Endocrinol*. 2012; 26:762–774. [PubMed: 22446104]
32. Seillet C, Rouquie N, Foulon E, Douin-Echinard V, Krust A, Chambon P, Arnal JF, Guery JC, Laffont S. Estradiol promotes functional responses in inflammatory and steady-state dendritic cells through differential requirement for activation function-1 of estrogen receptor alpha. *J Immunol*. 2013; 190:5459–5470. [PubMed: 23626011]
33. Cunningham MA, Wirth JR, Naga O, Eudaly J, Gilkeson GS. Estrogen Receptor Alpha Binding to ERE is Required for Full Tlr7- and Tlr9-Induced Inflammation. *SOJ Immunol*. 2014; 2:07. [PubMed: 25061615]
34. Cernetich A, Garver LS, Jedlicka AE, Klein PW, Kumar N, Scott AL, Klein SL. Involvement of gonadal steroids and gamma interferon in sex differences in response to blood-stage malaria infection. *Infect Immun*. 2006; 74:3190–3203. [PubMed: 16714546]
35. Jansen R, Batista S, Brooks AI, Tischfield JA, Willemsen G, van Grootheest G, Hottenga JJ, Milaneschi Y, Mbarek H, Madar V, Peyrot W, Vink JM, Verweij CL, de Geus EJ, Smit JH, Wright FA, Sullivan PF, Boomsma DI, Penninx BW. Sex differences in the human peripheral blood transcriptome. *BMC Genomics*. 2014; 15:33. [PubMed: 24438232]
36. Murphy AJ, Guyre PM, Pioli PA. Estradiol suppresses NF-kappa B activation through coordinated regulation of let-7a and miR-125b in primary human macrophages. *J Immunol*. 2010; 184:5029–5037. [PubMed: 20351193]
37. Dai R, McReynolds S, Leroith T, Heid B, Liang Z, Ahmed SA. Sex differences in the expression of lupus-associated miRNAs in splenocytes from lupus-prone NZB/WF1 mice. *Biol Sex Differ*. 2013; 4:19. [PubMed: 24175965]
38. Holm A, Andersson KE, Nordstrom I, Hellstrand P, Nilsson BO. Down-regulation of endothelial cell estrogen receptor expression by the inflammation promoter LPS. *Mol Cell Endocrinol*. 2010; 319:8–13. [PubMed: 20079402]
39. Panchanathan R, Shen H, Zhang X, Ho SM, Choubey D. Mutually positive regulatory feedback loop between interferons and estrogen receptor-alpha in mice: implications for sex bias in autoimmunity. *PLoS One*. 2010; 5:e10868. [PubMed: 20526365]
40. Matsuda KI, Yanagisawa M, Sano K, Ochiai I, Musatov S, Okoshi K, Tsukahara S, Ogawa S, Kawata M. Visualisation and characterisation of oestrogen receptor alpha-positive neurons expressing green fluorescent protein under the control of the oestrogen receptor alpha promoter. *Eur J Neurosci*. 2013; 38:2242–2249. [PubMed: 23601009]
41. Shen H, Panchanathan R, Rajavelu P, Duan X, Gould KA, Choubey D. Gender-dependent expression of murine Irf5 gene: implications for sex bias in autoimmunity. *J Mol Cell Biol*. 2010; 2:284–290. [PubMed: 20802013]
42. Panchanathan R, Liu H, Choubey D. Expression of murine Unc93b1 is up-regulated by interferon and estrogen signaling: implications for sex bias in the development of autoimmunity. *Int Immunol*. 2013; 25:521–529. [PubMed: 23728775]
43. Panchanathan R, Shen H, Bupp MG, Gould KA, Choubey D. Female and male sex hormones differentially regulate expression of Ifi202, an interferon-inducible lupus susceptibility gene within the Nba2 interval. *J Immunol*. 2009; 183:7031–7038. [PubMed: 19890043]
44. Smith S, Ni Gabhann J, McCarthy E, Coffey B, Mahony R, Byrne JC, Stacey K, Ball E, Bell A, Cunnane G, Doran MF, Molloy ES, Lee RZ, Harvey B, Kearns G, Jefferies CA. Estrogen receptor alpha regulates tripartite motif-containing protein 21 expression, contributing to dysregulated

- cytokine production in systemic lupus erythematosus. *Arthritis Rheumatol.* 2014; 66:163–172. [PubMed: 24449583]
45. Young NA, Wu LC, Burd CJ, Friedman AK, Kaffenberger BH, Rajaram MV, Schlesinger LS, James H, Shupnik MA, Jarjour WN. Estrogen modulation of endosome-associated toll-like receptor 8: an IFN $\alpha$ -independent mechanism of sex-bias in systemic lupus erythematosus. *Clin Immunol.* 2014; 151:66–77. [PubMed: 24525049]
  46. Meier A, Chang JJ, Chan ES, Pollard RB, Sidhu HK, Kulkarni S, Wen TF, Lindsay RJ, Orellana L, Mildvan D, Bazner S, Streeck H, Alter G, Lifson JD, Carrington M, Bosch RJ, Robbins GK, Altfeld M. Sex differences in the Toll-like receptor-mediated response of plasmacytoid dendritic cells to HIV-1. *Nat Med.* 2009; 15:955–959. [PubMed: 19597505]
  47. Berghofer B, Frommer T, Haley G, Fink L, Bein G, Hackstein H. TLR7 ligands induce higher IFN- $\alpha$  production in females. *J Immunol.* 2006; 177:2088–2096. [PubMed: 16887967]
  48. Weckerle CE, Niewold TB. The unexplained female predominance of systemic lupus erythematosus: clues from genetic and cytokine studies. *Clin Rev Allergy Immunol.* 2011; 40:42–49. [PubMed: 20063186]
  49. Tasker C, Ding J, Schmolke M, Rivera-Medina A, Garcia-Sastre A, Chang TL. 17 $\beta$ -estradiol protects primary macrophages against HIV infection through induction of interferon- $\alpha$ . *Viral Immunol.* 2014; 27:140–150. [PubMed: 24801776]
  50. Rodriguez-Garcia M, Biswas N, Patel MV, Barr FD, Crist SG, Ochsenbauer C, Fahey JV, Wira CR. Estradiol reduces susceptibility of CD4 $^{+}$  T cells and macrophages to HIV-infection. *PLoS One.* 2013; 8:e62069. [PubMed: 23614015]
  51. Cunningham MA, Naga OS, Eudaly JG, Scott JL, Gilkeson GS. Estrogen receptor alpha modulates Toll-like receptor signaling in murine lupus. *Clin Immunol.* 2012; 144:1–12. [PubMed: 22659029]
  52. Siracusa MC, Overstreet MG, Housseau F, Scott AL, Klein SL. 17 $\beta$ -Estradiol Alters the Activity of Conventional and IFN-Producing Killer Dendritic Cells. *J Immunol.* 2008; 180:1423–1431. [PubMed: 18209037]
  53. Douin-Echinard V, Laffont S, Seillet C, Delpy L, Krust A, Chambon P, Gourdy P, Arnal JF, Guery JC. Estrogen receptor alpha, but not beta, is required for optimal dendritic cell differentiation and [corrected] CD40-induced cytokine production. *J Immunol.* 2008; 180:3661–3669. [PubMed: 18322171]
  54. Delpy L, Douin-Echinard V, Garidou L, Bruand C, Saoudi A, Guery JC. Estrogen enhances susceptibility to experimental autoimmune myasthenia gravis by promoting type 1-polarized immune responses. *J Immunol.* 2005; 175:5050–5057. [PubMed: 16210608]
  55. Calippe B, Douin-Echinard V, Delpy L, Laffargue M, Lelu K, Krust A, Pipy B, Bayard F, Arnal JF, Guery JC, Gourdy P. 17 $\beta$ -Estradiol promotes TLR4-triggered proinflammatory mediator production through direct estrogen receptor alpha signaling in macrophages in vivo. *J Immunol.* 2010; 185:1169–1176. [PubMed: 20554954]
  56. Klein SL, Hodgson A, Robinson DP. Mechanisms of sex disparities in influenza pathogenesis. *J Leukoc Biol.* 2012; 67–73. [PubMed: 22131346]
  57. Relloso M, Aragonese-Fenoll L, Lasarte S, Bourgeois C, Romera G, Kuchler K, Corbi AL, Munoz-Fernandez MA, Nombela C, Rodriguez-Fernandez JL, Diez-Orejas R. Estradiol impairs the Th17 immune response against *Candida albicans*. *J Leukoc Biol.* 2012; 91:159–165. [PubMed: 21965175]
  58. Robinson DP, Lorenzo ME, Jian W, Klein SL. Elevated 17 $\beta$ -estradiol protects females from influenza A virus pathogenesis by suppressing inflammatory responses. *PLoS Pathog.* 2011; 7:e1002149. [PubMed: 21829352]
  59. Robinson DP, Hall OJ, Nilles TL, Bream JH, Klein SL. 17 $\beta$ -estradiol protects females against influenza by recruiting neutrophils and increasing virus-specific CD8 T cell responses in the lungs. *J Virol.* 2014; 88:4711–4720. [PubMed: 24522912]
  60. Papenfuss TL, Powell ND, McClain MA, Bedarf A, Singh A, Gienapp IE, Shawler T, Whitacre CC. Estriol generates tolerogenic dendritic cells in vivo that protect against autoimmunity. *J Immunol.* 2011; 186:3346–3355. [PubMed: 21317386]



61. Segerer SE, Muller N, van den Brandt J, Kapp M, Dietl J, Reichardt HM, Rieger L, Kammerer U. Impact of female sex hormones on the maturation and function of human dendritic cells. *Am J Reprod Immunol.* 2009; 62:165–173. [PubMed: 19694642]
62. Feldman I, Feldman GM, Mobarak C, Dunkelberg JC, Leslie KK. Identification of proteins within the nuclear factor-kappa B transcriptional complex including estrogen receptor-alpha. *Am J Obstet Gynecol.* 2007; 196 394.e1-11; discussion 394.e11.
63. Kalaitzidis D, Gilmore TD. Transcription factor cross-talk: the estrogen receptor and NF-kappaB. *Trends Endocrinol Metab.* 2005; 16:46–52. [PubMed: 15734144]
64. Nettles KW, Gil G, Nowak J, Metivier R, Sharma VB, Greene GL. CBP Is a dosage-dependent regulator of nuclear factor-kappaB suppression by the estrogen receptor. *Mol Endocrinol.* 2008; 22:263–272. [PubMed: 17932106]
65. Hasan UA, Zannetti C, Parroche P, Goutagny N, Malfroy M, Roblot G, Carreira C, Hussain I, Muller M, Taylor-Papadimitriou J, Picard D, Sylla BS, Trinchieri G, Medzhitov R, Tommasino M. The human papillomavirus type 16 E7 oncoprotein induces a transcriptional repressor complex on the Toll-like receptor 9 promoter. *J Exp Med.* 2013; 210:1369–1387. [PubMed: 23752229]
66. Xing D, Oparil S, Yu H, Gong K, Feng W, Black J, Chen YF, Nozell S. Estrogen modulates NFkappaB signaling by enhancing IkappaBalpha levels and blocking p65 binding at the promoters of inflammatory genes via estrogen receptor-beta. *PLoS One.* 2012; 7:e36890. [PubMed: 22723832]
67. Dai R, Phillips RA, Ahmed SA. Despite inhibition of nuclear localization of NF-kappa B p65, c-Rel, and RelB, 17-beta estradiol up-regulates NF-kappa B signaling in mouse splenocytes: the potential role of Bcl-3. *J Immunol.* 2007; 179:1776–1783. [PubMed: 17641044]
68. Dai R, Phillips RA, Karpuzoglu E, Khan D, Ahmed SA. Estrogen regulates transcription factors STAT-1 and NF-kappaB to promote inducible nitric oxide synthase and inflammatory responses. *J Immunol.* 2009; 183:6998–7005. [PubMed: 19890039]
69. Lasarte S, Elsner D, Sanchez-Elsner T, Fernandez-Pineda A, Lopez-Fernandez LA, Corbi AL, Munoz-Fernandez MA, Relloso M. Estradiol downregulates NF-kappaB translocation by Ikbkg transcriptional repression in dendritic cells. *Genes Immun.* 2013; 14:462–469. [PubMed: 23883819]
70. Smithson G, Couse JF, Lubahn DB, Korach KS, Kincade PW. The role of estrogen receptors and androgen receptors in sex steroid regulation of B lymphopoiesis. *J Immunol.* 1998; 161:27–34. [PubMed: 9647203]
71. 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.
72. Copley MR, Beer PA, Eaves CJ. Hematopoietic stem cell heterogeneity takes center stage. *Cell Stem Cell.* 2012; 10:690–697. [PubMed: 22704509]
73. Wang W, Dong L, Saville B, Safe S. Transcriptional activation of E2F1 gene expression by 17beta-estradiol in MCF-7 cells is regulated by NF-Y-Sp1/estrogen receptor interactions. *Mol Endocrinol.* 1999; 13:1373–1387. [PubMed: 10446910]
74. 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]
75. 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]
76. David A, Trigunait A, Macleod MK, Johnson AC, Marrack P, Jorgensen TN. Intrinsic autoimmune capacities of hematopoietic cells from female New Zealand hybrid mice. *Genes Immun.* 2014; 15:153–161. [PubMed: 24477163]
77. Schuettpelez LG, Link DC. Regulation of hematopoietic stem cell activity by inflammation. *Front Immunol.* 2013; 4:204. [PubMed: 23882270]
78. Kincade PW, Medina KL, Payne KJ, Rossi MI, Tudor KS, Yamashita Y, Kouro T. Early B-lymphocyte precursors and their regulation by sex steroids. *Immunol Rev.* 2000; 175:128–137. [PubMed: 10933598]

79. 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]
80. Welner RS, Pelayo R, Garrett KP, Chen X, Perry SS, Sun XH, Kee BL, Kincade PW. Interferon-producing killer dendritic cells (IKDCs) arise via a unique differentiation pathway from primitive c-kit<sup>hi</sup>CD62L<sup>+</sup> lymphoid progenitors. *Blood.* 2007; 109:4825–4931. [PubMed: 17317852]
81. Harman BC, Miller JP, Nikbakht N, Gerstein R, Allman D. Mouse plasmacytoid dendritic cells derive exclusively from estrogen-resistant myeloid progenitors. *Blood.* 2006; 108:878–885. [PubMed: 16507769]
82. Carreras E, Turner S, Frank MB, Knowlton N, Osban J, Centola M, Park CG, Simmons A, Alberola-Ila J, Kovats S. Estrogen receptor signaling promotes dendritic cell differentiation by increasing expression of the transcription factor IRF4. *Blood.* 2010; 115:238–246. [PubMed: 19880499]
83. Zhang QH, Hu YZ, Cao J, Zhong YQ, Zhao YF, Mei QB. Estrogen influences the differentiation, maturation and function of dendritic cells in rats with experimental autoimmune encephalomyelitis. *Acta Pharmacol Sin.* 2004; 25:508–513. [PubMed: 15066222]
84. Komi J, Lassila O. Nonsteroidal anti-estrogens inhibit the functional differentiation of human monocyte-derived dendritic cells. *Blood.* 2000; 95:2875–2882.
85. Kovats S. Estrogen receptors regulate an inflammatory pathway of dendritic cell differentiation: Mechanisms and implications for immunity. *Horm Behav.* 2012:254–262. [PubMed: 22561458]

Table 1

Expression of estrogen receptors by immune cells

Cell type	Human		Murine		References
	<i>ESR1</i> (ER $\alpha$ ) <sup>a</sup>	<i>ESR2</i> (ER $\beta$ )	<i>Esr1</i> (ER $\alpha$ )	<i>Esr2</i> (ER $\beta$ )	
B cell	yes (++++) <sup>b</sup>	yes (+++)	yes	yes	2,3,7
CD4 <sup>+</sup> T cell	yes (++)	yes (++)	yes	<i>c</i>	2,3,10
CD8 <sup>+</sup> T cell	yes (++)	yes (++)			2,3
NK cell	yes (++)	yes (++)	yes	yes	2,8
Plasmacytoid DC	yes (++)	yes (+++)	yes		2,6
Monocyte	yes (+)	yes (+)			2,3
Monocyte-derived DC	yes (++)	yes (+)			2
BM-derived DC			yes	yes	11,12
Splenic DC			yes	no	9
Inflammatory DC (CNS)				yes	14
Peritoneal Macrophage			yes	no	9,13
BM-derived Macrophage			yes	no	13
Hematopoietic stem cell	yes	yes	yes	no	18,20
Myeloid progenitor			yes	no	19

<sup>a</sup>“Yes” indicates either RNA or protein expression, depending on the study. “No” indicates that the RNA or protein was queried but not found.

<sup>b</sup> Plus (+) marks indicate relative amounts of RNA determined using quantitative methods in one study [ref 2].

<sup>c</sup> Empty cells indicate cell types for which actual data showing ER expression was not readily found in literature searches.