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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 (ERs) 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.

1. ER expression in immune cells

ER α and β proteins are members of the nuclear receptor super family encoded by the *ESR1* and *ESR2* genes, respectively [1]. Single ER chains form αα, ββ and αβ 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 (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 $CD4^+$ T cells, $CD8^+$ 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 ERα, and in some cases *Esr2* (encoding ERβ). Murine lymphocytes (B, T and NK cells) contain *Esr1* and ERα [7–10], and B and NK cells were reported to express ERβ protein [7,8]. Murine splenic DCs (including conventional and plasmacytoid DCs), as well as bone marrow-derived DCs, express *Esr1* and ERα but negligible *Esr2* and ERβ [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 ERβ [14], suggesting that ERβ 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].

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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. $CD34^+$ 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^+ c-kit^+ (LSK)], but not by fetal liver progenitors [18,19]. A recent study with highly purified murine HSCs (defined as LSK $CD150^+$ $CD48^-$) 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^- c-kit^+ $Flt3^+$) express *Esr1* but not *Esr2* [19]. Of note, data reported in the Immunological Genome Project (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 ERα and ERβ proteins, splice variants leading to truncated proteins have been described. For example, human macrophages predominantly express the N-terminal truncated ERα46 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 ERα. The pioneer factors found at >80% of ERα binding sites include FOXA1, PBX1, AP-2γ, TLE1, PBX1 and GATA3. ERα is preferentially recruited to DNA elements present in open chromatin, characterized by DNAse I hypersensitivity sites. ERα 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 ERα bound elements to transcription start sites [24]. Once bound, ERα 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-κB, SP1, AP-1, C/EBPβ) 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 ERα also can participate in transcriptional complexes [27]. The complex of ERα, 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 ERα that is associated via a palmitoylated site (Cys 447 in humans) with the cytosolic face of the plasma membrane. In the plasma membrane, ERα is localized to caveolae/lipid rafts by direct binding to

Table 1
Expression of estrogen receptors by immune cells.

Cell type	Human		Murine		Refs.
	ESR1 (ERα)[a]	*ESR2* (ERβ)	*Esr1* (ERα)	*Esr2* (ERβ)	
B cell	Yes (+++)[b]	Yes (+++)	Yes	Yes	[2,3,7]
$CD4^+$ T cell	Yes (++)	Yes (++)	Yes	[c]	[2,3,10]
$CD8^+$ 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]

[a] "Yes" indicates either RNA or protein expression, depending on the study. "No" indicates that the RNA or protein was queried but not found.
[b] Plus (+) marks indicate relative amounts of RNA determined using quantitative methods in one study (Ref. [2]).
[c] Empty cells indicate cell types for which actual data showing ER expression was not readily found in literature searches.