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The Immune System Is a Natural Target for Estrogen Action: Opposing Effects of Estrogen in Two Prototypical Autoimmune Diseases

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Analogous to other physiological systems, the immune system also demonstrates remarkable sex differences. Although the reasons for sex differences in immune responses are not precisely understood, it potentially involves differences in sex hormones (estrogens, androgens, and differential sex hormone receptor-mediated events), X-chromosomes, microbiome, epigenetics among others. Overall, females tend to have more responsive and robust immune system compared to their male counterparts. It is therefore not surprising that females respond more aggressively to self-antigens and are more susceptible to autoimmune diseases. Female hormone (estrogen or 17β-estradiol) can potentially act on all cellular subsets of the immune system through estrogen receptor-dependent and -independent mechanisms. This minireview highlights differential expression of estrogen receptors on immune cells, major estrogen-mediated signaling pathways, and their effect on immune cells. Since estrogen has varied effects in female-predominant autoimmune diseases such as multiple sclerosis and systemic lupus erythematosus, we will mechanistically postulate the potential differential role of estrogen in these chronic debilitating diseases.

Keywords: estrogen, autoimmune, SLE, MS, signaling, immune cell

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INTRODUCTION

Although the principal function of sex steroid action is to regulate reproductive functions, studies in diverse fields have unequivocally established that sex steroids also act on diverse non-reproductive tissues include immune, central nervous, cardiovascular, and skeletal systems, as well as cells from liver, skin, and kidneys (1–5). In this brief review, we will discuss estrogen effects on the immune cells, the estrogen-specific receptor expression on cells of the immune system, and key estrogen-receptor mediated-signaling pathways involved. In addition, we will analyze the differential response of estrogen in two typical female-predominant autoimmune diseases.

ESTROGEN REGULATION OF CELLS OF THE INNATE AND ADAPTIVE IMMUNE SYSTEM

There is now an enormous amount of literature on estrogen's effects on the cells of the innate immune system [neutrophils, macrophages/monocytes, natural killer cells, dendritic cells (DC)], and the adaptive immune system (T and B cells). These aspects have been comprehensively covered in many reviews (5–7) and hence are beyond the scope of this minireview. Estrogens have been shown to regulate neutrophil numbers and functions that include chemotaxis, infiltration, production of superoxide anion and myeloperoxidase, induction of chemokines (cytokine induced neutrophil chemoattractants such as CINC-1, CINC-2β, and CINC-3, monocyte chemoattractant protein-1), and cytokines (e.g., TNF-α, IL-6, IL-1β) (8–10). **Table 1** shows key selected genes that estrogen regulates in the cells of the immune system. Our laboratory has recently shown that *in vivo* estrogen-treated C57BL/6 mice have increased splenic neutrophils comparable to that noticed in female autoimmune-prone MRL/lpr or C57BL/6-lpr (11). Estrogens can also alter macrophage function by regulating chemotaxis, phagocytic activity, and induction of cytokines, iNOS, and nitric oxide (12–16). Estrogen can also enhance differentiation of immature *DCs* into mature functional DCs, and regulate the expression of cytokines and chemokines such as IL-6, IL-10, CXCL8, and CCL2 (17, 18). Overall, multiple studies have demonstrated that estrogens can affect innate immune cell signaling (19–21).

Estrogen has been shown to modulate all subsets of T cells that include $CD4^+$ (Th1, Th2, Th17, and Tregs) and $CD8^+$ cells (32–35). Extensive studies have demonstrated that estrogen modulates IFNγ-secreting *Th1 cells* by enhancing IFNγ expression in both human and mice (23–25), which are potentially mediated by direct interaction of ER with Estrogen-response element (ERE) in the promoter region of the *Ifn*γ gene (24) and/or up-regulating Th-1-specific transcription factor T-bet (25, 36). ERα-deficient mice have decreased $IFN\gamma^+$-secreting cells in lymph nodes, suggesting estrogen-driven Th1 cell responsiveness is dependent on ERα-mediated signaling (37). Estrogen's effect on Th2 cells and its prototypic cytokine, IL-4 is less marked, either having no effect (25, 38, 39) or stimulatory effect of estrogen on IL-4 secretion and *GATA-3 expression* (26) or a positive correlation between menstrual estrogen cycle levels and IL-4 (40). Interestingly, high levels of estrogen (e.g., pregnancy level) are known to skew the immune response from Th1 (IFNγ) to Th2 (IL-4) (41–43). The effects of estrogen on Th17 subset have also been recently reported, albeit with varied response to estrogen depending on the experimental conditions. In periodontal ligament cells culture, addition of estrogen enhances IL-1β-mediated IL-17F production (44). In adult cystic fibrosis male mice, estrogen increases the severity of pneumonia, in part by increased Th17-regulated inflammation (45). However, it has also been shown that estrogen deficiency in postmenopausal women is associated with increased IL-17A levels (46). Estrogen also promotes the expansion and frequency of Treg cells, which play a critical role in downregulating immune responses (28, 30) and upregulating the expression of FoxP3, PD-1, and CTLA-4 via ERα-mediated signaling (27–30). Protective effects of estrogen in autoimmune conditions such as MS and RA are believed to be due to a combined result of estrogen-mediated Treg expansion and activation (27, 47, 48).

Estrogen can also have profound effects on B cell differentiation, activity, function (49, 50), and survival by increasing expression of genes such as *cd22, shp-1, bcl-2,* and *vcam-1* (31) Estrogen has been shown to increase plasma cell and autoantibody producing cells numbers (49, 51). Although signaling by either ERα or β has shown to alter B cell maturation, ERα engagement has been shown to be critical for autoimmunity (52).

The outcome of response of estrogen on the immune system can vary depending upon the level of estrogens, cell type, activation state of cells, local environment, and the experimental context. In many of these studies, it is unclear if estrogenic effects are mediated through ER-dependent or -independent pathways. Nonetheless, the estrogen-mediated effects are apparent in all major innate and adaptive immune cells.

TABLE 1 | List of key selected genes that are regulated by estrogen in cells of innate and adaptive immune system.

Immune cell	List of genes	Reference
Neutrophil	CINC-1, CINC-2β, CINC-3, TNFα, IL-6, IL-1β	(8–10)
Macrophage	iNOS, NO, IL-6, TNFα	(12–16)
Dendritic cells	IL-6, IL-10, CXCL8, CCL2, TGFβ, IL-23, IL-12	(17, 18, 22)
Th1	IFNγ	(23–25)
Th2	IL-4	(26)
Tregs	FoxP3, PD-1, CTLA-4	(27–30)
B cells	Immunoglobulin, CD22, SHP-1, Bcl-2, VCAM-1	(31)

ESTROGEN RECEPTOR EXPRESSION IN THE CELLS OF THE IMMUNE SYSTEM

Estrogen-mediated signaling is a result of fine-tuned balance between two distinct receptors ERα (NR3A1) and ERβ (NR3A2) that are encoded by *ESR-1* and *ESR-2* genes expressed on human chromosomes 6 and 14, respectively (53). These receptors act as ligand-activated transcription factors and, therefore, directly regulate a broad range of estrogen-responsive genes. The biochemical similarities and differences between ERα and ERβ are depicted in **Figure 1A**. Eventhough both ERs have comparable affinity to estrogen and recognize the same ERE, they may have distinct, non-overlapping or even antagonist effects. There are different parameters that determine the overall effect of estrogen receptor-mediated signaling. These factors include: (i) differential distribution and expression of ERs in various cells and tissues, (ii) homo or hetero dimerization of the receptor, (iii) distinct splice variant ER isoforms, (iv) diverse signaling pathways triggered, (v) interaction with specific co-activators/-repressors, (vi) transactivation, (vii) physiological or pathological states, and (viii) local tissue milieu, among others.

In most cells of the immune system ERα is expressed, which includes hematopoietic cells, bone marrow, thymus stromal cells and thymocytes (6, 7, 56–59), and murine splenic DC and

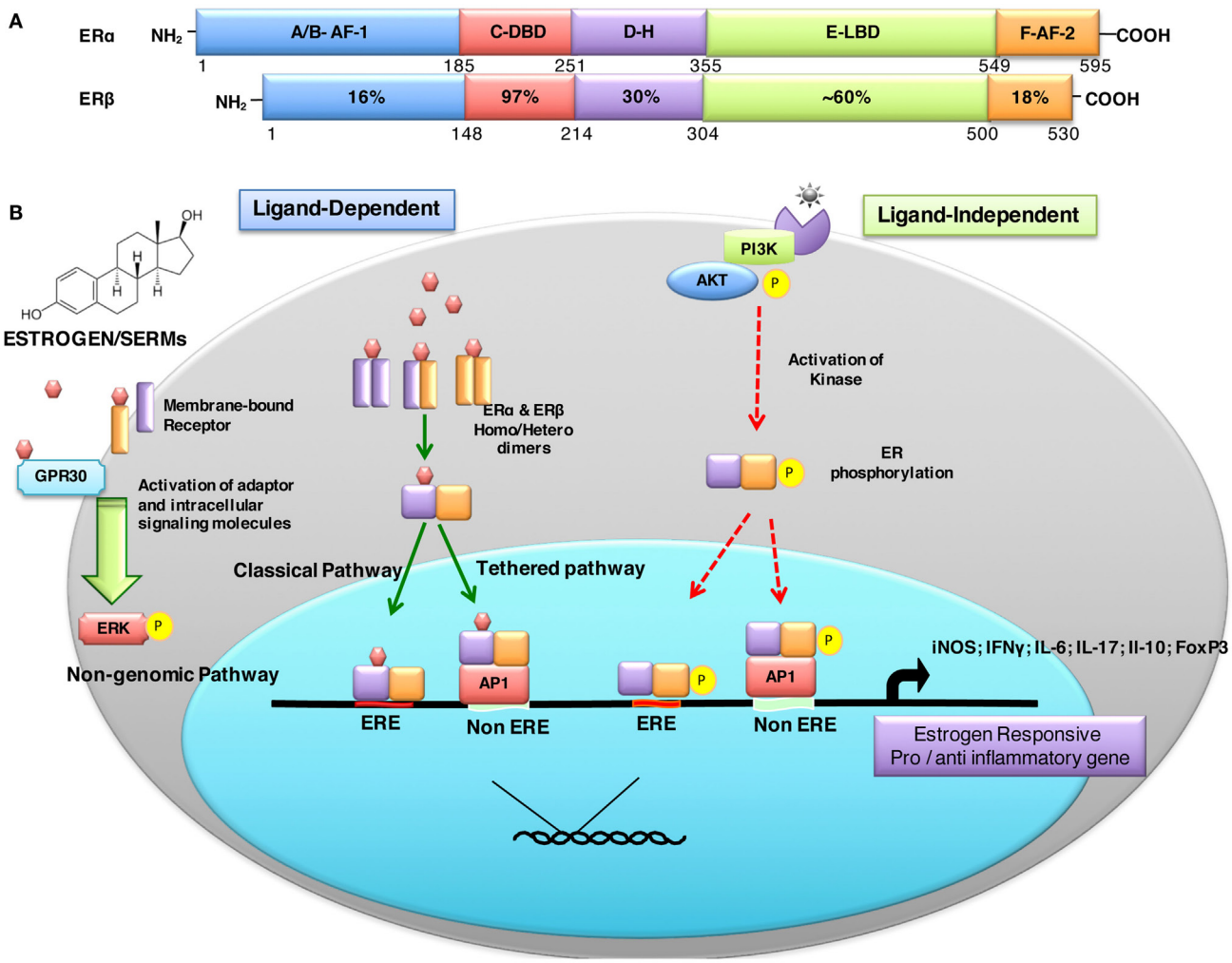

FIGURE 1 | Structural description and percent sequence homology of human ERα and ERβ and schematic representation of estrogen receptor ligand-dependent and ligand-independent signaling. **(A)** shows comparison of human estrogen receptor α (595 aa) and a shorter estrogen receptor β (530 aa). These receptors are evolutionarily conserved and have five distinct structural and functional domains: DNA-binding domain (DBD; C domain), hinge domain (D), ligand-binding domain (LBD; E/F domain), and two transcriptional activation function domains AF-1 (in A/B domain) and AF-2 (in F domain). The binding of ligand (estrogens) to E domain results in conformational changes in the receptor (homo/hetero dimerization). The receptor dimer than translocates inside nuclei with the help of D domain. This domain is also important for post-translational modifications of receptor by acetylation, lipophilic moieties, and ubiquitination. The C domain then recognizes and binds to estrogen-response element (ERE) in DNA. AF-2 region interacts with co-regulatory proteins in ligand-dependent pathway. However, AF-1 region is activated in ligand-independent manner (54). **(B)** ER-mediated signaling occurs in a ligand-dependent (green arrows) and ligand-independent (red arrows). The ligand-dependent pathway is triggered by binding of either endogenous hormone or a synthetic compound to the ligand-binding domain of ERs in the cytosol. Different ligands induce unique conformational changes of ERs, and receptor dimerization (homodimers: ERα:ERα or ERβ:ERβ or heterodimer: ERα:ERβ), which then translocate into nuclei and bind to specific EREs (consisting of a 5-bp palindrome with a 3-bp spacer; *GGTCAnnnTGACC*) in the regulatory regions of estrogen responsive genes. This is also called "*classical*" signaling pathway. In "*tethered*" signaling pathway, ligand-activated ERs interact with other transcription factor complexes and bind to non-EREs by attaching to other transcription factors and not with ERE directly. In third ligand-dependent "*non-genomic*" pathway, ligand interacts with plasma membrane-bound ERs via palmitoylation on cysteine447, which results in activation of cytoplasmic signaling pathways, such as protein kinase C (PKC). In *ligand-independent signaling* pathway, there is phosphorylation/activation of ERs by other active signaling cascades in a cell (55). This activation results in both direct ERE and non-ERE dependent genomic actions. Abbreviation: Akt, protein kinase B; AP-1, activator protein 1; ERE, estrogen-response element; ER, estrogen receptor; ERK, extracellular signal-regulated kinase; FoxP3, fork box 3; GRP30, an orphan G-protein coupled receptor 30; iNOS, inducible nitric oxide synthase; PI3K, phosphatidylinositol-4, 5-bisphosphate 3-kinase; P, indicates phosphorylation.

peritoneal macrophages (60). Whereas ERβ has a more restricted cellular expression and is preferentially expressed in thymus and spleen of human mid-gestational fetus (61), lymphocytes in human lymph nodes, rat thymocyte and stromal cells (58), murine bone marrow, and thymus (62, 63). Among T lymphocytes, $CD4^+$ cells have more ERα levels compared to ERβ whereas $CD8^+$ cells have low expression of both receptors. B cells, unlike CD4 cells, have more ERβ than ERα (64). Effect of ERα on the immune system is generally considered to be developmentally more prominent than ERβ (37) since ERα-deficient mice exhibit hypoplastic

thymus and spleen (65, 66), increased immature double-positive thymocytes ($CD4^+CD8^+$) and decreased $CD4^+CD8^-$ cells. The balance between ERα and ERβ to maintain a physiological state is underlined by the observations that mice lacking ERα have increased immune complex-mediated glomerulonephritis, proteinuria, infiltration of B cells in kidney, damage of tubular cells, and presence of serum anti-DNA antibodies (66, 67). The natural varied expression of ERs in different tissues and cell types, and during maturity, finely balances the overall outcome of estrogen-mediated immune responses.

ESTROGEN RECEPTOR-MEDIATED CELL SIGNALING

In large part, estrogen mediates its effects by binding to specific estrogen receptors and triggering distinct signaling pathways to regulate a broad range of estrogen responsive genes. ER-mediated signaling can be broadly classified as either ligand dependent or ligand independent (**Figure 1B**). Additionally, post-translational modifications of ERs also affect ER signaling and biological functions. These include: phosphorylation stimulates signaling; glycosylation is important for ER localization; acetylation enhances ER-DNA binding activity, hormone sensitivity, and transcriptional activity; sumoylation favors ERα-dependent transcription; nitrosylation reduces DNA binding ability; ubiquitination promotes degradation; myristoylation and palmitoylation affect cross-talk of ERs with membrane proteins, trafficking, as well as signal transduction (68). It is thus not surprising that varied ER-mediated effects in different experimental and clinical situations may in part be due to local differential post-translational modifications of ER.

ESTROGEN AND AUTOIMMUNE DISEASES

The pathogenesis of autoimmune diseases remains unclear despite extensive research over a few decades. However, multiple factors that regulate autoimmune diseases have been identified. These include: genetics; epigenetics (miRNA, methylation, and histone deacetylation); infections; and external and internal environmental triggers including hormones and microbiome. A majority of autoimmune diseases occur predominantly in women, a feature also noted in many animal models of autoimmune diseases (5, 69). Initially, it was presumed that the sex bias is related to differences in sex steroids, but it is now evident that several other factors contribute to the sex bias of autoimmune diseases that include X chromosomal abnormalities, X-chromosomal inactivation, and fetal microchimerism. The effects of sex hormones (such as estrogens) on autoimmune diseases cannot be generalized and is context/disease-dependent. It is not surprising that the outcome of estrogen-mediated autoimmune responses is different among autoimmune diseases since estrogens affect all cells of the immune system, and the triggering and pathogenic mechanisms are varied among different diseases. This aspect of differential estrogen-mediated effects in autoimmune diseases, in two classical female-predominant diseases: an organ-specific [multiple sclerosis (MS)] and a non-organ-specific autoimmune disease (SLE), are highlighted below.

Estrogen and MS

Multiple sclerosis (MS) and its experimental model (EAE) are characterized by the presence of myelin antigens reactive $CD4^+$ T cells in the CNS, demyelination of axons resulting in axonal death, and altered CNS function. The women to men ratio for disease prevalence ranges from 2.3 to 3.5:1 (70). The exact mechanism of this female predisposition of MS remains unknown. Yet intriguingly, the female sex hormone estrogen is protective in MS. Estrogens have been shown to have anti-inflammatory and neuroprotective effect in MS and EAE. Estrogen through principally ERα-dependent mechanism decreases autoantigen-specific pro-inflammatory biomolecules (such as IFNγ, TNFα, IL-17, iNOS, and MCP-1), and inhibits inflammation and demyelination (32, 47, 48, 71). ERβ agonist diarylpropionitrile (DPN) protects oligodendrocytes by increasing endogenous myelination (72). A recent report has demonstrated that estrogen protects gray matter atrophy in EAE (73). In addition in the EAE model, estrogen inhibits $CD4^+$ T cells expansion, increases proportions of Tregs and $CD4^+CD8^-$ suppressor T cells (74), increases T cell apoptosis (75), and markedly alters expression pattern of 315 genes in spinal cord tissue of mice protected from EAE (76). In pregnant EAE mice, there is reduced CNS pathology and decreased TNFα and IL-17 production when compared to non-pregnant controls (77). The rate of relapse in females increases postpartum at a phase when there is a marked decrease in estrogen levels when compared to pregnancy levels (78, 79). *In vivo* estriol treatment promotes generation of tolerogenic DCs with increased activation markers (CD80 and CD86), inhibitory costimulatory markers (PD-L1, PD-L2, B7-H3, and B7-H4), and increased anti-inflammatory (IL-10 and TGFβ) and decreased pro-inflammatory (IL-12, IL-23, and IL-6) cytokine mRNA expression (22).

Estrogen and SLE

The female:male susceptibility ratio for SLE is 9–20:1 (69, 80–82). Although the precise effect of estrogens in human SLE is not clear, unlike MS, a majority of studies have shown that estrogen is not protective in SLE. Rather, studies in a number of relevant animal models for SLE show that estrogen may have opposite effects. Several studies have shown that estrogen enhances severity and flares of disease in both human and animal models (83, 84). Estrogen enhances anti-double-stranded DNA antibody and IgG, IgM production by PBMCs, and serum from patients with SLE (85). Estrogen increases reactivity to exogenous antigens and also increases expression of endogenous autoantigens, e.g., human endogenous retrovirus (HERV) (86, 87), which molecularly mimics RNP antigens, and is increased in SLE patients (88). Estrogen also promotes systemic inflammation and induction of B cell activating factor and IFN signature genes (89). Over 50% of the genes that are altered during menstrual cycle are also markedly altered in SLE patients when compared to healthy controls (90). Tumor necrosis factor receptor superfamily member (TNFRSF14) also called Herpes virus entry mediator (HVEM), which interacts with B and T lymphocyte attenuator (BTLA) and downregulates lymphocyte activation and homeostasis

(91), is altered in normal females when compared to males, as well as in SLE patients when compared to normal controls (90). Although during a menstrual cycle, there is estrogen-mediated increased expression of TNFRSF14 mRNA in PBMCs, in SLE patients, there is decreased TNFRSF14 mRNA, which results in only partial immune suppression by BTLA culminating in overall immune enhancement (91, 92).

Altered ER expression in SLE patients and different murine lupus models potentially results in their hyper-reactivity to estrogen (93, 94). There is increased ERα and decreased ERβ mRNA expression in PBMCs of SLE patients (94). Increased ERα expressing $CD4^+$ and $CD8^+$ T cells and $ER\alpha^+$ DCs and macrophages in estrogen-treated autoimmune $SNF_{(1)}$ mice as compared to control $DBF_{(1)}$ mice has also been reported (95). It is evident from different studies that ERα-mediated signaling is required for exaggeration of lupus disease (95, 96). Furthermore, ERα polymorphism is also reported in SLE patients (97, 98). Different risk alleles susceptibility loci, lupus-susceptibility genes, and SNPs have also been identified in SLE patients such as interferon regulatory factor (IRF5); HLA-DR2 and HLA-DR3, Ifi202 of Ifi200-family, PTPN22, CTLA4, STAT4 and BANK1, TLR 7, 8, 9, etc. (99–103). Nuclear antigens activate TLR7- and TLR9-mediated IRF5 activation, which increases IFNα secretion in SLE patients (104, 105). Estrogen signaling via ERα also upregulates IRF5 mRNA (106). Recent reports have implied an important role for TLR8 and TLR9 in checking TLR7-mediated spontaneous autoimmunity in mice (107). In addition, X-linked TLR8 dosage also plays critical role in increased susceptibility of females to SLE (108). Single copy of TLR8 in 564Igi *Tlr7/9*$^{-/-}$ mice is not enough for autoantibody production, granulopoeisis, and *Ifn-I* expression (108). Although unclear how estrogen promotes lupus in relevant EAE, it is conceivable that estrogen may have complex multicellular effects that include altered ER signaling, stimulating pro-inflammatory cytokines from Th and other cells, augmenting pathogenic autoantibodies by favoring localized action of Th2 cells, aberrant TLR-mediated signaling, enhancing autoantigen presentation, downregulating regulatory apparatus, and dysregulating microRNA expression.

CONCLUSION

There is now a wealth of data that affirms estrogen regulates various facets of the immune system via complex molecular mechanisms. Although both MS and SLE are autoimmune diseases, they differ in many respects that include target pathological organs, triggering and pathogenic mechanisms, predominant effector cell type(s), genetics, and epigenetics among others. Given that estrogen affects all cells of the immune system as well as non-lymphoid tissues (e.g., vascular endothelial cells) that are in proximity of target tissues, it is conceivable that estrogen will have different local effects. Therefore, it is not surprising that estrogen has been shown to have different effects in various autoimmune diseases. While sex hormones may play a role in sex-differences in autoimmune diseases, clearly sex hormones alone do not exclusively contribute to this sex differential susceptibility. Precisely why females are more susceptible to autoimmune diseases continues to be an intriguing area of investigation. It is therefore important to fully understand the complex interaction of estrogen in context-specific situations. It is plausible that estrogen may have varied epigenetic effects (microRNAs, histone modifications, and/or acetylation) (109) and different ER-mediated post-translational modifications in different diseases such as in MS and SLE. Indeed, we have shown that estrogen induces signature miRNA expression in lymphocytes (14) and accelerates the expression of lupus-associated miRNAs in lupus-prone mice (110). It is also likely that the microbiome in MS and SLE are different. Microbiome can affect sex hormone production and vice versa (111, 112), which in turn affects systemic immune responses. Local estrogen and response levels altered by microbiome may be different in these two autoimmune diseases, an aspect that merits investigations. It is conceivable that any immune cell (innate and adaptive) that expresses ERα and/or ERβ can potentially respond to estrogen in a context-dependent fashion, which will affect the outcome of immune or autoimmune responses. Given spatial and temporal expression of ERs, it is important to have a comprehensive knowledge and evaluation of ER expression in a particular tissue before designing potential ER-targeted therapies. In relation to personalized medicine, with the advent of highly sensitive molecular arrays, metagenomics and bioinformatics, it is plausible to integrate these techniques to better predict the estrogen-mediated immunomodulatory effects in a disease-specific fashion.

AUTHOR CONTRIBUTIONS

DK and SA designed the work, drafted and revised the work, and finally approved the version to be published and agree to be accountable for all aspects of the work.

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