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Review

# Sex hormone signaling and regulation of immune function

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## SUMMARY

Immune responses to antigens, including innocuous, self, tumor, microbial, and vaccine antigens, differ between males and females. The quest to uncover the mechanisms for biological sex differences in the immune system has intensified, with considerable literature pointing toward sex hormonal influences on immune cell function. Sex steroids, including estrogens, androgens, and progestins, have profound effects on immune function. As such, drastic changes in sex steroid concentrations that occur with aging (e.g., after puberty or during the menopause transition) or pregnancy impact immune responses and the pathogenesis of immune-related diseases. The effect of sex steroids on immunity involves both the concentration of the ligand and the density and distribution of genomic and nongenomic receptors that serve as transcriptional regulators of immune cellular responses to affect autoimmunity, allergy, infectious diseases, cancers, and responses to vaccines. The next frontier will be harnessing these effects of sex steroids to improve therapeutic outcomes.

## INTRODUCTION

In the seven years since the US National Institutes of Health implemented the policy that sex as a biological variable (SABV) be factored into the research design, analyses, and reporting in vertebrate animal and human studies,[1] the field of immunology has made progress in reporting use of both sexes in preclinical and clinical studies.[2] Considering SABV in research studies is not the same as rigorously studying sex differences.[3] Sex differences refer to biological differences between males and females based on sex chromosome complement, the development of reproductive tissues, and concentration of sex steroids. Sex differences are often analyzed as a binary variable (i.e., male/XY vs. female/XX), but intersex individuals (i.e., individuals born with reproductive characteristics of both males and females) as well as Turner syndrome (i.e., XO individuals) and Klinefelter syndrome (i.e., XXY individuals) patients highlight that sex occurs on a continuum, which should be considered in the context of immune function. Separate but complementary are gender differences that reflect the social-cultural construct of being a man, woman, or transgender person.[4] In many individuals, biological sex (male or female) matches the subject's gender (man or woman). In other individuals, biological or birth sex is incongruent with gender, and gender affirming care may be sought at different stages of the life course and with different courses of treatment (i.e., gender affirming hormone treatment with or without gender affirming surgery). The implications of being transgender has not been thoroughly evaluated in the context of immune function (Figure 1).

Sex differences in immune cell function can occur because of differences in the expression of sex chromosome-encoded genes on either the X or Y in immune cells or differences in the expression of autosomal genes in immune cells because of sex steroid receptor signaling and epigenetic modifications.[5] Sex differences in immune cell function have been observed across various innate and adaptive immune cell types, both in the resting state and in the context of diseases, such autoimmune diseases, cancer, allergy, asthma, and infectious diseases.[5] These sex differences in immunity not only impact the pathogenesis of diseases but also the responses to disease treatments including immunomodulatory therapies, vaccines, and immune checkpoint inhibitors.[6]

Sex differences in immune function are dynamic, changing over the life course and during different reproductive stages of life (e.g., after puberty, during pregnancy, and during the menopause transition). During critical developmental and reproductive stages of life, there are profound changes that occur in the concentrations of sex steroids (e.g., estrogens, androgens, and progestins) and signaling through sex steroid receptors (Figure 1). Because sex steroids are known modulators of sex differences in immune function, we seek to identify the molecular and cellular pathways regulated by sex steroid receptor signaling in immune cells, with consideration of how this contributes to sex, age, and reproductive state differences in immune function and outcomes of diseases. A better understanding of the transcriptional signaling pathways mediated by sex steroid receptors in immune cells could provide targetable pathways

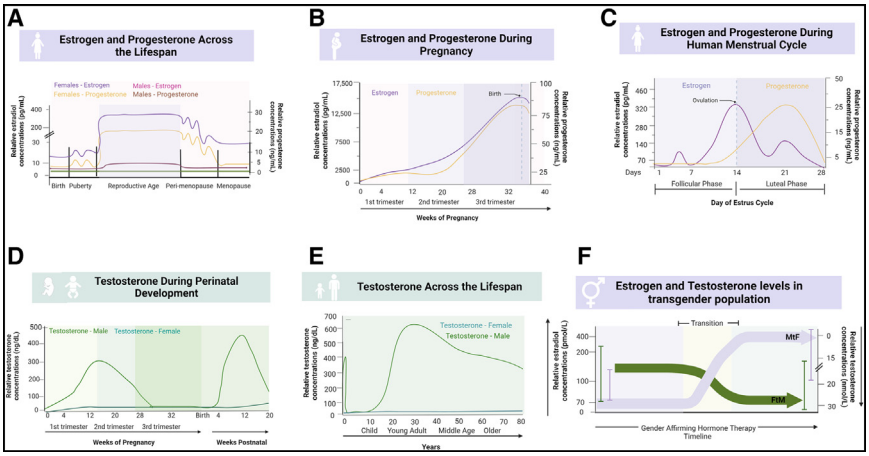


**Figure 1. Concentrations of sex steroid across the lifespan**

Concentrations of estrogen (i.e., the primary biologically active 17β-estradiol) and progesterone vary across the lifespan (A) in both human females and males, with circulating concentrations changing over the course of pregnancy (B), and the menstrual cycle (C) in humans. Concentrations of androgens (i.e., primarily testosterone) in females and males over the course of the human lifespan, with increases occurring during perinatal development (i.e., mini puberty) (D) and during adolescence (E) in males. Among transgender females (M to F), relative concentrations of estrogens (i.e., 17β-estradiol) increase and concentrations of androgens (e.g., testosterone) decrease after receipt of gender affirming hormone therapy. Among transgender males (F to M), relative concentrations of androgens increase, and concentrations of estrogens decrease after receipt of gender affirming hormone therapy (F).

for novel treatments for diseases ranging from autoimmunity and cancer to infectious diseases.

## ESTROGENS AND ESTROGEN RECEPTOR SIGNALING

Estrogens are derived from androgens and include estrone (E1), estradiol (E2), and estriol (E3). Estradiol is the primary biologically active estrogen secreted by the ovaries during reproductive years, while estrone, primarily secreted from the adrenal glands, increases in concentrations during reproductive senescence. Estriol is solely produced by the placenta during pregnancy. Across the life course, males and females have differential concentration of circulating estrogens with females have higher concentration than males prior to reproductive senescence (i.e., menopause in humans) (Figure 1). There are several synthetic estrogens that can act as either agonists or antagonists to the estrogen receptor (ER). Ethinyl estradiol is the most utilized synthetic steroidal form in oral contraceptive pills. In addition to pregnancy prevention, other FDA-approved clinical use for estrogens includes female hypogonadism, menopause associated osteoporosis, acne, and vulgaris. Less common are consideration of the immunomodulatory properties of estrogens, which have been used with limited success for treatments for autoimmune diseases (e.g., multiple sclerosis[7]) and COVID-19.[8,9]

ERs, both nuclear and membrane bound, have been identified in various innate and adaptive immune cell types (Figure 2).[10] The nuclear ERs (nERs) include ERα and ERβ, whereas the membrane-bound ER is the G-protein-coupled estrogen receptor (GPER) or GPR30. ERs are members of the nuclear receptor super family and contain three functional domains that include transactivation domain, DNA-binding domain, and ligand-binding domain.[11,12] Signaling through the ER can elicit long-term and rapid signal transduction via genomic or ligand-dependent or nongenomic ligand-independent signaling, respectively.

The rapid, nongenomic signaling via GPER, now known as membrane-initiated steroid signaling (MISS), has been elucidated using synthetic E2 conjugates that are unable to enter the nucleus.[13–15] In the GPER signaling pathway, the second messenger signaling transduction system is initiated via intracellular calcium and cAMP induction, phospholipase C activation leading to PI3K/AKT and ERK protein kinase pathway activation.[16] ERβ generally acts as an agonist in response to E2 treatment and ERα and ERβ can regulate distinct sets of genes and produce varying effects. Both the expression and the function of ERβ varies based on tissue and cell types.[17,18] In some cases, ERα and ERβ have overlapping functions, while in others, they are opposing.[19] Out of 228 genes, only 38 (17%) genes are regulated by both ERα and ERβ with E2 treatment.[20] ER signaling regulates cell proliferation factors, growth factors, cytokines (e.g., interferons, IL-6, IL-1, VEGF, amphiregulin, and TGF-β), receptors and signaling pathways (e.g., NF-κB, STAT, TGF-β, and TNF), and transcription factors and coregulators (e.g., c-Fos, c-Myc, Myb, and JunB).[10,21,22] ER signaling can transcriptionally regulate immune cell functions and with dramatic changes in concentrations of estrogens over the female life course, activation ER signaling in immune cells is altered.

## ER SIGNALING IN NEUTROPHILS

ER signaling regulates the acute inflammatory and innate immune response of neutrophils. *In vivo* and *in vitro* studies illustrate that estrogen concentrations influence ERα and ERβ expression in neutrophils.[23] In humans, neutrophils isolated from premenopausal women collected during the follicular phase have greater ERα and ERβ expression than neutrophils isolated during the ovulatory phase of the menstrual cycle.[24] Premenopausal women have greater numbers of neutrophils in circulation compared with postmenopausal women.[24] In rats, administration of E2 or selective ER agonists (e.g., 4,4′,4′′-(4-propyl-[1H]-pyrazole-1,3,5-triyl) trisphenol [PPT] and 2,3-bis(4-hydroxyphenyl)-propionitrile [DPN]) upregulates genes involved in inflammation and extracellular matrix remodeling, including genes that encode for 12-lipoxygenase, fibulin-1, furin, and calgranulin B.[25] During infection with *Pseudomonas aeruginosa*, both ERα and ERβ signaling hinders neutrophil killing capacity in female compared with male mice, which is reversed by administration of ER antagonists.[26] Whether ER signaling in neutrophils impacts outcomes of other infectious diseases has not been explored. Most studies only show evidence of E2 increasing neutrophil frequencies in infected tissues,[27] but whether this is caused by infected cells producing chemokines that attract

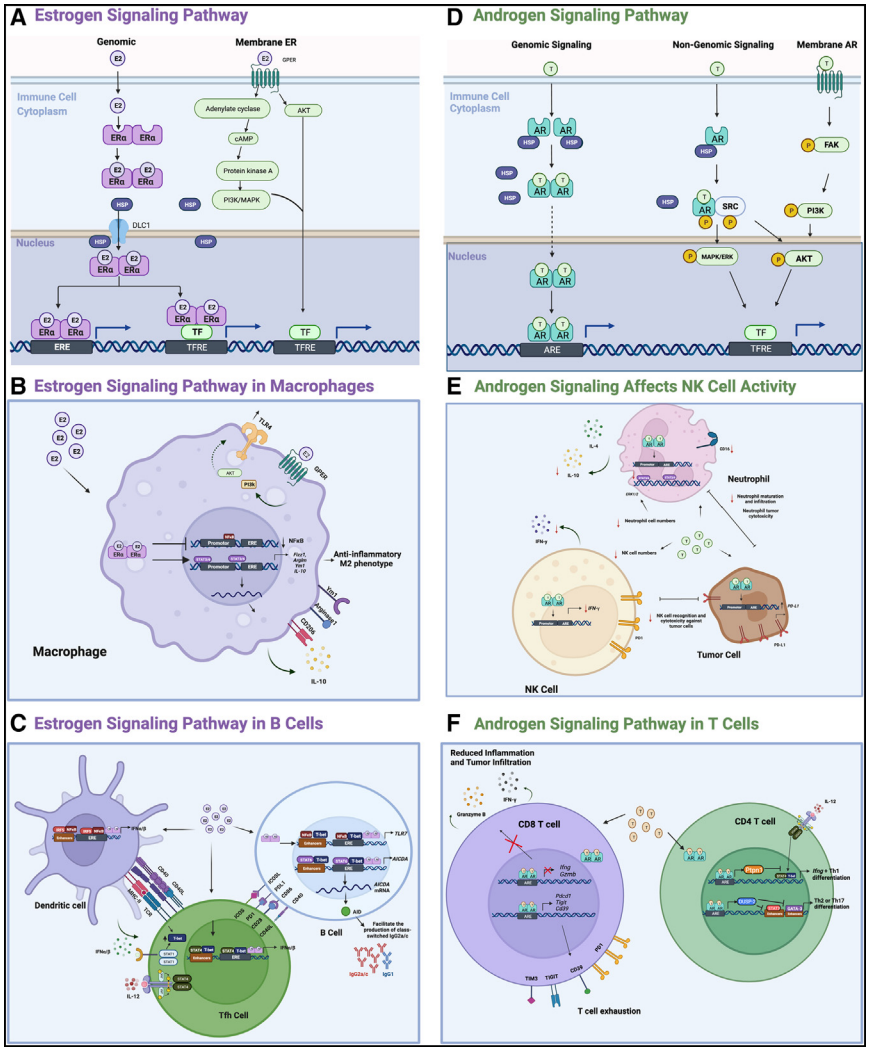


**Figure 2. Estrogen and androgen signaling pathways in innate and adaptive immune cells**

(A) Estrogens, including 17β-estradiol (E2), can bind to both intracellular (i.e., genomic) and membrane-bound (i.e., nongenomic) estrogen receptors (ERs) to cause transcriptional changes in immune cells.

(B) E2 bound to genomic ERs translocate to the nucleus of macrophages to bind to estrogen response elements (EREs) on the genes that encode for several inflammatory and anti-inflammatory proteins, which can, for example, increase secretion of IL-10 and promote an anti-inflammatory phenotype.

(C) Genomic ER signaling in dendritic cells, follicular helper T cells, and B cells regulates co-receptor engagement, cytokine production, and activation of genes that encode for the proteins (e.g., TLR7 and AID) that underlie class-switch recombination and somatic hypermutations to regulate the antibody repertoire, which in mice can alter the ratio of IgG subclasses.

(D) Androgens, including testosterone (T), can bind to either intracellular (i.e., genomic and nongenomic) and membrane-bound androgen receptors (ARs) to regulate activity in immune cells.

(E) Testosterone (T), for example, can bind to ARs in neutrophils, NK cells, and tumor cells. The activation of ARs and binding to androgen response elements (AREs) on genes (e.g., IFN-γ) in NK cells upregulates the expression of immunoregulatory receptors (e.g., PD-1) as well as reduces cytokine production, cytotoxic activity, and killing of tumor cells.

(F) Intracellular AR signaling in $CD8^+$ T cells can repress IFN-γ and granzyme B activation and promote markers of exhaustion. In $CD4^+$ T cells, T binding to intracellular ARs cause the AR complex to translocate to the nucleus, bind to AREs on genes that repress differentiation into Th1, Th2, and Th17 subtypes.

neutrophils or if ER signaling within neutrophils is causing these effects remains understudied.

Neutrophil infiltration into a tumor microenvironment is regulated by ER signaling. In the tumor microenvironment, neutrophils comprise a significant portion of infiltrating immune cells. In tumor-bearing mice, E2 facilitates neutrophil infiltration into the tumor microenvironment,[28] highlighting that estrogens modify cellular communication to influence neutrophil activity. In human renal cell carcinoma, infiltrating neutrophils promote renal cell carcinoma through ERβ signaling and VEGFα/HIF2α pathways.[29] In a spinal cord injury model, as compared with saline-treated mice, mice treated with E2 have reduced white matter spinal cord injury, cell death, and edema, which correlates with reduced infiltration of neutrophils to the site of injury.[30] Myeloperoxidase activity and the expression of cytokines and chemokines, including IL-1β, IL-6, TNF, and MCP-1 are reduced in spinal cord tissue in E2-treated mice compared with saline-treated mice, which is partially reversed by administration of the ER antagonist ICI 182,780.[30] Together, these data highlight that ER signaling dampens inflammation in part through direct effects of neutrophils, and this contributes to both sex differences and menstrual cycle changes in neutrophils activity.

## ER SIGNALING IN NATURAL KILLER CELLS

Natural killer (NK) cells express both ERα and ERβ, and signaling through these receptors modulates NK cell activity.[31] Across several mouse strains, gonadectomy and replacement with exogenous E2 reduces NK cell cytotoxicity.[32] In ERα-deficient as well as wild-type mice, E2 reduces NK cell cytotoxicity, suggesting that other receptors or signaling mechanisms are involved.[33] E2 treatment increases the number of NK cells but reduced cytotoxicity[34] in part by altering the expression of genes involved in cellular cytotoxicity and proliferative capacity, including genes that encode CD94 and IFN-γ.[35] In humans, combined estrogen and medroxyprogesterone hormone replacement therapy administered to healthy postmenopausal human females is associated with reduced NK cell cytotoxicity and synthesis of IL-2 and IFN-γ.[36]

The impact of ER signaling in NK cells on disease outcomes has been most well studied in the context of cancer. Greater concentrations of E2 and signaling through ERα reduces NK-cell-induced death in breast cancer cell lines by increasing granzyme B inhibitors and proteinase inhibitor 9, whereas treatment an NK cell line (NK92) with tamoxifen impairs cytotoxicity.[37] Patients with cervical cancer tend to have poor infiltration of NK cells into the tumor microenvironment, with E2 reducing NK cell cytotoxicity of tumor cells and altering CD107a expression.[38] Taken together, *in vivo* and *in vitro* studies highlight that ER signaling suppresses NK cell function. Additional research into the impact of ER signaling on NK cell function is required in the context of diseases other than cancers.

## ER SIGNALING IN MACROPHAGES AND MONOCYTES

ERα and ERβ along with membrane-bound G-protein-coupled receptor (GPR30/GPER-1) are expressed in macrophages[39] (Figure 2). Broadly, ER signaling promotes anti-inflammatory phenotypic macrophages by regulating cytokine production, phagocytosis, and chemotaxis.[40] In macrophages, ER signaling modulates NF-κB nuclear translocation through ERα, but not ERβ, by impairing transcriptional activity of p65 and preventing NF-κB intracellular localization during an LPS-induced inflammatory response.[41] E2 inhibits macrophage activation during *in vitro* LPS stimulation by inhibiting matrix metalloproteinase-9.[42] E2 and P4 increase survival of undifferentiated macrophages and macrophage-like PMA-differentiated U937 cells through upregulation of ERα, ERβ, and progesterone receptor (PR) mRNAs.[43] Membrane-bound G-protein-coupled GPR30/GPER-1 also has anti-inflammatory effects in macrophages through cell surface regulation of Toll-like receptor 4 (TLR4)[39] (Figure 2). ERα-deficient murine macrophages stimulated with LPS have increased TNF release illustrating that ERα, but not ERβ, signaling is responsible for inhibiting proinflammatory cytokine production.[33] Exogenous E2 treatment during the resolution phase of inflammation reduces the proinflammatory phenotype in macrophage (RAW 264.7) cells and accelerates resolution of LPS-induced inflammation through IL-10 activation,[44] which also is important during tissue remodeling and immunomodulation.[45] In BV-2 murine microglial cell lines stimulated with LPS, E2 reduces NO, inducible nitric oxide synthase (iNOS) expression, and COX-2 expression, and enhances TNF mRNA expression,[46] suggesting that the inflammatory and anti-inflammatory effects of ER signaling in macrophages is cell-type dependent.

ER signaling contributes to sex differences in disease outcomes. For example, E2 enhances IL-4-induced M2 gene expression in bone-marrow-derived and alveolar macrophages in female mice following respiratory allergen challenge.[47] Using a cutaneous wound repair model, E2 reduces proinflammatory gene expression through ERα signaling in macrophages that promotes wound healing.[48] In an imiquimod-induced psoriatic inflammatory mouse model, ovariectomized mice have greater concentrations of inflammatory cytokines, including IL-17A, and IL-1β, which is reversed by exogenous administration of E2 and subsequently abolished when ERs are specifically knocked out in neutrophils and macrophages using *esr1*f/f*esr2*f/f-*lysm*-Cre+ mice.[49] In mouse models of hepatocarcinoma tumors, E2 reduces tumor growth via ERβ-driven inhibition of the JAK1/STAT-6 signaling pathway in macrophages.[50] In contrast, in non-small cell lung cancer, greater ERα expression is associated with greater numbers of infiltrating macrophages through binding of ER to estrogen response elements (ERE) activating CCL2/CCR2.[51]

Microglia express ER and have a prominent role in immune surveillance and initiation of neuroinflammatory response in the central nervous system (CNS).[52] In microglia, ER signaling promotes anti-inflammatory responses[52] that are neuroprotective during CNS injury.[53] Generally, signaling through ERβ and not ERα modulates microglia function. Treatment of experimental autoimmune encephalomyelitis (EAE) mice with ERβ agonists reduces the severity of clinical disease and mortality.[54,55] Together, these studies highlight that ER signaling in most macrophage populations reduces inflammation and immune response, including in diverse disease models.

## ER SIGNALING IN DENDRITIC CELLS

Dendritic cells (DCs) and progenitor subsets express both ERα and ERβ, and signaling through these receptors modulates DC differentiation and activity. *In vitro* and *in vivo* studies illustrate that ER signaling, predominantly ERα, is essential for regulating differentiation, cytokine production, and activity.[56–59] For example, E2 signaling through ERα, but not ERβ, promotes GM-CSF stimulated DC differentiation.[56] Incubation of bone-marrow-derived cells in Flt3L-supplemented medium devoid of estrogens or in the presence of an ER antagonist (ICI 182,780) reduced DC differentiation.[60] *In vivo* studies using ERα- or ERβ-deficient mice show that estrogen-dependent activation of ERα is necessary for DC differentiation from bone marrow and cytokine production.[59] In response to E2 activation of ERα, plasmacytoid DC cells (pDCs) have greater TLR-mediated cytokine secretion, which is consistent with the observation that estrogen replacement therapy administered to postmenopausal women enhances TLR7 and TLR9 stimulated IFN-α production by pDCs.[56] The stimulatory effects of E2 on pDC production of type I IFNs is abolished in ERα-deficient mice.[56] The impact of sex steroid signaling also is implied by the observation of sex differences in pDC cytokine production following TLR stimulation. Among both humans and mice, females have greater IFN-α production following TLR7 stimulation, which at least in mice is reversed in the absence of ERα.[61]

To date, few studies have characterized the functional role of ER signaling in DCs in the context of disease outcomes. In a murine model of EAE, in the absence of ERα in DCs, mice become resistant to IFNβ treatment and produce more neurotoxic $CD4^+$ effector T cells, resulting in greater neurodegeneration.[62] Also in the EAE mouse model, using ERβ ligands, adoptive transfer, and cell-specific ERβ knockout, it is apparent that ERβ signaling in DCs is neuroprotective by reducing DC expression of TNF in the CNS.[63] Taken together, these data highlight that ER signaling directly mediates DC development and activation to affect cellular function and disease outcomes.

## ER SIGNALING IN T CELLS

Both ERα and ERβ are expressed in T cells. In general, $CD4^+$ T cells express higher levels of ERα than ERβ, while $CD8^+$

T cells express similar levels of both receptors.[64–66] In mice, 85% of circulating CD4$^+$ T cells expressed ERα, while less than 1% express ERβ.[66] As a result, the CD4$^+$ T cell literature focuses predominantly on ERα signaling mechanism and function.

In autoimmune disease mouse models, ERα and ERβ signaling typically have opposing functions, with ERα signaling being proinflammatory and ERβ signaling being anti-inflammatory in CD4$^+$ T cells. In mouse models of colitis, ERα knockout in CD4$^+$ T cells reduces secretion of proinflammatory cytokines, such as TNF, IL-6, IL-17, and IFN-γ.[67–69] In murine colitis models, loss of ERα signaling reduces inflammation and colon pathology and increases frequencies of regulatory T cells (Tregs) in the lamina propria.[67,68] In humans with rheumatic heart disease, ERα signaling in CD8$^+$ T cells potentiates prothymosin alpha expression along with expression of granzyme and perforin, which is reversed by treatment with ERα antagonists.[70] In most autoimmune disease models, ERα signaling in T cells is proinflammatory; there are, however, some autoimmune disease for which ERα signaling in T cells is anti-inflammatory.[71–75] Therefore, the impact of ER signaling might be autoimmune-disease-specific but collectively contributes to sex differences in susceptibility to diverse autoimmune diseases.[67,68,70,76,77]

In both mice and humans, ERβ signaling in T cells tends to suppress inflammatory T cells responses. In mouse colitis models, knocking out or blocking ERβ signaling in T cells reduces FoxP3 expression and promotes T cell infiltration in the intestines and intestinal inflammation.[68,77] Patients with Crohn's disease, ulcerative colitis, or inflammatory bowel disease have decreased expression of ERβ in T cells both in the ileal mucosa and the periphery.[68,78] In EAE models, ERβ signaling reduces CD4$^+$ and CD8$^+$ T cell accumulation in the CNS, the production of IFN-γ, IL-17, and iNOS, and disease severity.[54,79] In patients with multiple sclerosis, circulating Tregs express less ERβ than cells from healthy controls.[80] T cells from patients with severe systemic lupus erythematosus (SLE) also express less ERβ than cells from healthy controls.[81] While less is known about ER signaling in γδ T cells, one study found that in the absence of ERβ signaling, interstitial cystitis of the bladder ensues and is caused by an influx of γδ T cells.[82]

ER signaling in T cells also is a critical mediator of sex differences in allergic inflammation and infectious diseases. In asthma patients, isolation and treatment of Th2 cells with an ERα agonist reduces apoptosis and increases secretion of IL-5 and IL-13.[83] In asthma patients, females have greater frequencies of Th17 cells that secrete greater concentrations of IL-17 than cells from male patients.[84] In mice, E2 treatment of Th17 cells enhances IL-17 and IL-23R expression; the specific ER through which E2 signals, however, is not identified.[84] CD4$^+$ T cells isolated from murine vaginal tissue, utilize ERα signaling for the expression of IL-17.[85] In contrast, others have shown that ERα can actively bind to and inhibit RORγT, a transcriptional regulator of Th17 differentiation.[86]

With regard to infectious disease, ERα signaling in CD4$^+$ T cells can limit activation of HIV.[87] In a murine model of *Streptococcus pneumoniae*, ERβ signaling in female mice enhances numbers of Tregs and reduces disease severity. Although ERβ activation and increased the numbers of Tregs does not influence bacterial burden, the reduced pulmonary inflammation improves disease outcomes[88] (Figure 3). During coxsackievirus B3 infection, which causes viral myocarditis to a greater extent in male than female mice, ERβ signaling decreases Treg frequencies in the heart.[89] Differential effects of ERβ signaling on infectious disease outcomes likely depend on whether the microbe is a virus or bacteria, the role of CD4$^+$ T cell subsets in protection versus pathology, and whether it is males or females who are more susceptible to adverse outcomes.

Cancer studies reveal a prominent role of ERα and ERβ signaling in T cells. In lung and cervical tumor samples, ERα signaling is associated with decreased infiltration of CD4$^+$ and CD8$^+$ T cells into the tumor microenvironment.[90,91] ERα directly interacts with FoxP3 to promote its expression.[91] In a murine model of mammary cancer, a mutation in ERβ reduces infiltration of CD4$^+$ and CD8$^+$ T cells and concentrations of IFN-γ in the tumor microenvironment, resulting in increases in tumor size.[92] Because many cancers can dysregulate sex steroid hormone concentrations and expression of sex steroid receptors in tumor cells, bidirectional communication and regulation of these pathways contribute to complexities in interpretation.[90,91,93]

## ER SIGNALING IN B CELLS

In humans and mice, ERs are highly expressed in B cells and ER signaling regulates proliferation, differentiation, and functionality of B cells in homoeostatic and diseased states.[94–96] At homeostasis, *in vitro* treatment of human peripheral blood B cells with 2-Methoxyestradiol (2-ME), an end-metabolite of E2, increases the expression of CD138 and CD27.[97] In mice, treatment of female splenic B cells with E2 decreases the expression of CD80 on B220+ cells and apoptosis, which is time and dose dependent.[97,98] In a mouse model of SLE, E2 treatment of female splenic B cells co-cultured with pDCs and stimulated with CpG indirectly improves B cell viability by increasing the expression of costimulatory molecules CD40 and CD86 on pDCs.[99] By influencing the expression of these necessary surface receptors, estrogen regulates the activation state of B cells (Figure 2).

In addition to influencing the expression of surface receptors, ER signaling affects intracellular signaling for proliferation, differentiation, and activation. Activation-induced deaminase (AID) is the enzyme crucial for antibody production[100] by deaminating cytosines at immunoglobulin loci thereby causing somatic hypermutation (SHM) and class-switch recombination (CSR). ER signaling regulates the expression and activity of AID through EREs in the promoter region of human AID.[100,101] Estradiol treatment of isolated murine female splenic B cells stimulated with IL-4 and LPS (i.e., inducers of AID) increases AID mRNA and protein expression due to direct binding of ER dimers to ERE in the AID promoter.[100] Estrogenic effects also are observed in Apobec3b, -3f, and -3g, indicating that ER signaling is conserved within the DNA deaminase family, which also can be inhibited by treatment with tamoxifen (i.e., an estrogen antagonist).[100] Diffuse large B cell lymphomas treated with the ER inhibitor, fulvestrant, have differential expression of key cell-cycle genes including those that encode for CDC6, CDC20, KIF20A, STIL, and TOP2A.[102] Furthermore, 2-ME-treated B cells downregulate Pax5 and Bcl-6 (i.e., differentiation repressors) and upregulate IRF4, Bcl-2, Blimp-1, and XBP-1 mRNA expression compared with vehicle-treated cells, resulting in increased numbers of CD138$^+$ plasma cell.[97] E2 also inhibits the production of

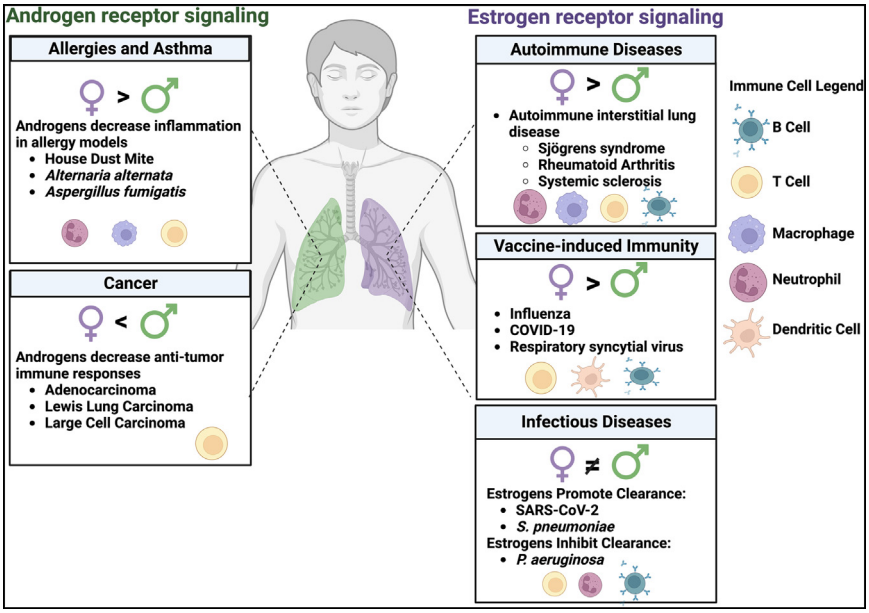


**Figure 3. Testosterone and estrogen receptor signaling in diverse cell types underlies sex differences in respiratory disease outcomes**

Sex differences are reported for several diseases that impact the respiratory tract, including allergies, asthma, cancers, autoimmune diseases, and infectious diseases. Greater circulating concentrations of testosterone are associated reduced pulmonary inflammation and anti-tumor immunity in males compared with females. In contrast, greater concentrations of estradiol and signaling through ER are associated with greater susceptibility to autoimmune diseases but also greater vaccine-induced immunity and protection against respiratory infections among females compared with males.

anti-inflammatory IL-10 in activated marginal zone B cells.[103] In human B cells, AID regulation of CSR following stimulation with either LPS, LPS and IL-4, LPS and IFN-γ, or LPS and TGF-β enhances production of IgG3, IgG1, IgG2a, or IgG2b/IgA, respectively.[100] In humans and mice, either E2 or 2-ME treatment increases total IgG antibody production in peripheral blood and splenic B cells, respectively.[97,98] These data illustrate that ER signaling regulates the molecular pathways associated with antibody production, which likely contributes to how females have greater antibody responses than males.

The severity and progression of autoimmune diseases, such as SLE, involve ER signaling and increased levels of the estrogenic metabolite, 16a-hydroxyestrone.[104–107] Mice deficient in ERα are prone to a lupus-like phenotype.[108] Treatment of peripheral blood mononuclear cells (PBMCs) with E2 increases anti-DNA antibodies and IgG secretion by B cells in SLE patients compared with untreated controls.[109] In a SLE mouse model, Src family tyrosine kinase deficient mice ($Blk^{-/-}$) have increased proportions of B-1 cells that produced increased low-affinity anti-dsDNA autoantibodies.[110] E2 directly acts on splenocytes to increase IgM and IgE production.[111] The duration of exposure to E2 has differential effects in B cells. A short-term E2 exposure increases the number of Ig producing cells in intact and ovariectomized mice, whereas long-term E2 exposure induces anti-dsDNA autoantibodies and renal Ig deposition.[109] The development of these DNA-associated autoantibodies is TLR9 dependent in B cells.[112] Development of autoantibodies in SLE relies on ERα-dependent IFN-α signaling in B cells. E2 enhances IFN-α signaling activation by downregulating the expression of microRNAs (i.e., let-7e-5p, miR-98-5p, and miR-145a-5p) that can directly target and regulate the expression of IKKε, subsequently promoting the activation of IFN-α signaling in B cells. The expression of these microRNAs is lower whereas IKKε is greater in female compared with age-matched male mice.[113]

Among humans, as compared with adult males, adult females develop almost two times as much antibody following vaccination against influenza, yellow fever, rubella, measles, mumps, hepatitis A and B, herpes simplex 2, rabies, smallpox, dengue viruses, and SARS-CoV-2.[114,115] Following vaccination in mice, adult females have greater numbers of germinal center (GC) B cells, more SHM in splenic GC B cells, and greater vaccine-specific IgG and neutralizing antibody responses, including CSR IgG2 than males.[116–118] Aging and reduced concentrations of E2 are associated with reduced vaccine-induced immunity and protection in females among both humans and mice.[117] Ovariectomy of adult female mice reduces, whereas treatment of ovariectomized female mice with exogenous E2 increases vaccine-induced antibody responses.[117] In humans, the concentration of circulating E2 is positively correlated with virus-specific neutralizing antibody responses.[117] Future studies must consider how hormone replacement therapies impact vaccine-induced antibody responses and the molecular pathways involved.

## ANDROGENS AND ANDROGEN RECEPTOR SIGNALING

In males, the first surge of testosterone occurs during fetal development between the first and second trimester and by the third trimester, testosterone concentrations drop to levels found in females (Figure 1). This is known as a mini puberty and facilitates the development of reproductive tissues and brain differentiation.[119] During the first few months following birth, testosterone concentrations in males surge once more to levels comparable to those during adolescent puberty.[119–121] During adolescence in males, serum testosterone secretion continues to rise, peaking in early to mid-20 years of age, and then gradually declining over the course of the male lifespan.[122,123] Females typically have very-low androgen levels throughout development. Conditions such as polycystic ovarian syndrome (PCOS), however, cause increased concentrations of testosterone in females.[124,125]

Testosterone can either be enzymatically aromatized into estrogens to signal through the ER or reduced into other androgens, such as dihydrotestosterone, to signaling through the androgen receptor (AR). For genomic signaling, the AR resides in the cytosol, and in the presence of androgen, the AR complex

translocates to the nucleus where it directly binds the DNA at androgen response elements (AREs) to promote gene expression.[126] For nongenomic signaling, after binding androgen, the AR can form a complex with proto-oncogene tyrosine-protein kinase (SRC). The AR-SRC complex can then phosphorylate MAPK, ERK, or AKT leading to the phosphorylation of various transcription factors that can then alter gene expression[127] (Figure 2).

## AR SIGNALING IN NEUTROPHILS

AR signaling regulates the number of neutrophils *in vivo* based on mouse models utilizing castrated males, AR knockout (ARKO) mice, and mice with mutation in the AR gene (*Tmf*), with all models illustrating that the lack of either androgens or AR signaling results in reduced circulating neutrophil counts (i.e., neutropenia) that can be restored with exogenous androgens in castrated mice, but not in ARKO mice.[128] Deletion of the AR gene impairs the proliferative capacity and maturation of neutrophils and reduces concentrations of proinflammatory cytokines (e.g., IL-1β, IL-6, and TNF-α) and chemokines (e.g., CCL2, CCL3, CCL4, CXCL1, CXCL4, and CXCL7) compared with wild-type mice.[128] AR signaling also alters neutrophil production of granulocyte colony-stimulating factor (G-CSF) via activation of ERK1/2 and reduces inhibitory binding of PIAS3 to STAT3 in neutrophils,[128] which supports the observation that active STAT3 signaling results in neutropenia.[129] Testosterone treatment of human neutrophils reduces proinflammatory cytokine production, anti-microbial activity, and oxidative stress responses.[130] In humans, young adult females with hyperinsulinemia hyperandrogenism that suffer from PCOS have greater neutrophil counts compared with young adult females without PCOS. The effects of PCOS on neutrophils is eliminated with antiandrogen treatment (i.e., flutamide), highlighting that greater androgen concentrations independent of sex alter neutrophil counts and function.[131]

The effects of AR signaling on neutrophils impact disease and tumor progression. In mouse models of melanoma, castration, AR-deficiency (i.e., *Tfm* mice), and intact males treated with anti-androgens (e.g., flutamide) have reduced tumor burden and progression from increased neutrophil maturation and infiltration compared with respective control mice.[132] In castrated, but not AR-deficient, male mice treated with exogenous testosterone have greater tumor growth and reduced neutrophil numbers and activity, indicating that loss of testosterone impairs neutrophil anti-tumor response.[132] Neutrophils isolated from prostate cancer patients undergoing androgen deprivation therapy have reduced frequencies, expression of CD16, and cellular cytotoxicity.[132] In other cancer models, androgens and AR signaling increase hepatic metastases tumor cell growth and neutropenia resulting in hepatic neutrophil accumulation in males.[133] Co-culture of renal cell carcinoma cells with neutrophils causes infiltrating neutrophils to upregulate AR signaling and alter c-Myc signaling pathways in renal cell carcinoma cells.[29] In a rodent model of acute bacterial prostatitis (i.e., uropathogenic *Escherichia coli*), treatment of gonadectomized male rats with testosterone not only increases neutrophil recruitment and accumulation but also promotes an anti-inflammatory neutrophil phenotype through elevated IL-10 and TGF-β expression, which results in prolonged bacterial inflammation and delayed recovery compared with gonadectomized male rats.[134]

## AR SIGNALING IN NK CELLS

NK cells express ARs.[135] NK cell number and activity are functionally different between sexes, with males having greater NK cell frequencies than females.[136] In contrast, NK cells from females are functionally more active, secreting greater concentrations of IFN-γ, which is regulated by epigenetic modulation of X-linked genes in NK cells.[137] The impact of AR signaling on NK cell numbers and functions is most well characterized in prostate cancer due to the central role of ARs in carcinogenesis, which results in targeted hormone therapeutics, such as androgen deprivation therapy as standard care.[138]

During development of prostate cancer, there is greater NK infiltration in tumors as compared with control prostate tissue, with AR signaling transcriptionally regulating NK inhibitory ligand LLT1 in castration-resistant prostate cancer cells.[135] AR enhances NK cell-tumor killing through reduced programmed death ligand 1 (PD-L1) expression.[139] Either AR knockdown or administration of androgen antagonist (i.e., enzalutamide) reduces PD-LI expression and facilitates NK-cell-mediated killing of bladder cancer.[139] In hepatocellular carcinoma, the chemotherapy, cisplatin enhances NK cell cytotoxicity by downregulating AR activity and UL-16 binding protein 2 expression.[140] AR signaling suppresses IL-12A expression through binding to promotor regions affecting transcription and repression of NK cytotoxicity.[140]

## AR SIGNALING IN MONOCYTES AND MACROPHAGES

AR signaling in macrophages has been most well characterized in models of cancer and tumor progression, where macrophages have critical function in the initiation and progression of cancers, including prostate cancer. Co-cultures of prostate epithelial cells with macrophages *in vitro* induces AR-induced inflammatory CCL4-STAT3 activation and subsequent downregulation of tumor suppressor genes p53/PTEN to facilitate prostate tumor growth.[141] Conditional knockout of AR in macrophages in prostate tumorigenesis mouse models causes fewer preneoplastic prostatic intraepithelial neoplasia lesions as compared with wild-type mice.[141] AR signaling in the macrophage-like THP-1 cell line is critical for prostate cancer cell line migration and invasion *in vitro*, in part by increasing triggering receptor expressed on myeloid cells-1 (TREM-1) signaling and expression of its downstream cytokines as well as upregulating activity of IL-10 and markers of tissue residency in THP-1 and monocyte-derived macrophages.[142] These data support that AR signaling in macrophages promotes prostate cancer growth through transcriptional regulation of cellular responses.

In mouse models of experimental autoimmune myocarditis, AR suppression increases numbers of anti-inflammatory macrophages and cytokine production, reduces STAT3/SOCS3 signaling, and causes inflammation during acute disease.[143] In other disease models, including allergic asthma and lung inflammation, AR has opposite effects where androgen (e.g., dihydrotestosterone) administered to castrated male mice enhances anti-inflammatory alveolar macrophage phenotypes

and reduced lung inflammation.[144] Monocyte/macrophage AR knockout (AR$^{flox}$*lysm*Cre) reduces lung inflammation and numbers of eosinophils recruited to the site of inflammation in male, but not female mice.[144] Induction of asthma using a mixed allergen challenge is improved through AR signaling, which regulates airway hyperresponsiveness and inflammation.[145] AR signaling in monocytes and macrophages contributes to sex differences in disease outcomes, which can be beneficial by dampening inflammation caused by allergy or asthma or detrimental for controlling tumorigenesis.

## AR SIGNALING IN T CELLS

The influence of AR signaling on T cells begins with their development in the thymus. Global AR knockout increases thymic weight and thymopoesis, in which there are greater numbers of double negative, double-positive, and single-positive T cells in the absence of AR signaling.[146,147] The thymic effects of AR signaling are dependent on epithelial cell rather than T cell AR signaling.[146,148] In thymic epithelial cells, AR signaling upregulates the expression of the autoimmune regulator (AIRE) gene that promotes the expression of self-antigen for the purpose of negative selection.[149] The sex bias in AIRE expression leads to enhanced development of immune tolerance. In the absence of AR signaling in mice, T cells have more robust TCR signaling, leading to increased positive selection and frequencies of T cells in the periphery.[146,147] The fact that AR signaling inhibits positive selection for T cells may underlie the predisposition of females for inflammatory diseases and autoimmunity.[150] With positive selection for more T cells migrating into the periphery, there is an increased risk of T cells reacting to self-antigens. These data, in addition to data from mice infected with influenza A virus,[151] highlight that AR signaling both directly and indirectly dampens T-cell-mediated inflammation.

Most studies demonstrate that AR signaling has a suppressive effect on T cell function and promotes tolerance, especially in the context of cancer. Androgen deprivation therapy is the most common treatment for incurable prostate cancer[152] as a means for increasing the activity of CD8$^+$ T cells. In a murine bladder carcinoma model, the selective knockout of AR in CD8$^+$ T cells promoted CD8$^+$ T cell-tumor infiltration and reduced tumor size.[153] Use of AR antagonists or AR knockouts in murine cancer models increase CD8$^+$ T cell proliferation and secretion of IFN-$\gamma$, TNF, and granzyme B.[154–156] In mice, AR bind to the open chromatin regions associated with granzyme B and IFN-$\gamma$ to directly repress expression.[154] In murine CD8$^+$ T cells, AR signaling inhibits the expression of the transcription factor TCF-1, which promotes the expansion, persistence, and stemness of CD8$^+$ T cells.[155]

AR signaling suppression of CD8$^+$ T cell function is observed in humans. In primary tumors from Her-2-positive breast cancer patients, AR expression in tumors is inversely correlated with CD8$^+$ immune cell infiltration into the tumor.[157] In human colorectal tumor samples, AR expression corelates with exhaustion markers (e.g., PD-1, TIGIT, and CD39) in CD8$^+$ cells[158] (Figure 2). The AR antagonist flutamide can inhibit anti-tumor immune function, in part because flutamide is a nonsteroidal antagonist that has off-target effects by signaling through GABA.[159] These studies demonstrate that AR signaling in T cells suppresses anti-tumor activity, which is therapeutically targetable.

The suppressive effects of AR signaling in T cells, particularly in CD4$^+$ T cells, are observed in the context of autoimmunity and allergic inflammation. In CD4$^+$ T cells, AR signaling prevents differentiation of naive T cells into Th1 or Th2 cells, while promoting Treg development.[160] In a murine *Alternaria alternata* allergy model, AR signaling increases the Treg/Th2 ratio and decreases airway inflammation.[160] Treatment of CD4$^+$ T cells with testosterone inhibits Th1 differentiation by preventing IL-12 induced STAT4 phosphorylation[161] (Figure 2). In a house dust mite allergy model, selective knockout of AR in CD4$^+$ T cells results in greater frequencies of T cells in the lungs, which express more Ki67 and the effector cytokines IL-4, IL-5, and IL-13.[162] Androgens also suppress Th2 differentiation and the formation of Th2 memory.[163] Global AR knockout in mice reduces numbers of Tregs in tissues, including visceral adipose tissue.[164] The treatment of PBMCs isolated from healthy human females with DHT enhances the expression of FoxP3, the transcription factor associated with Tregs.[165] AR signaling alters CD4$^+$ T cells differentiation and function to promote and anti-inflammatory state, which can either be beneficial or detrimental depending on the etiology of the disease.

## AR SIGNALING IN B CELLS

B cells express AR.[166,167] Most studies have defined the role of AR signaling in B cells in the context of autoimmune diseases. Use of murine models, including global AR knockout, B-cell-specific AR knockout, *Tfm* AR-mutant mice, and castrated wild-type male mice, reveals that the absence of AR signaling increases the frequency of B cells in the bone marrow compared with wild-type mice.[168] The increased frequency of bone marrow B cells in castrated male mice can be reversed with dihydrotestosterone treatment. Isolation of B cells from mice that have AR selectively deleted in B cells, and treatment with IL-7 results in decreased Fas protein expression, caspase-3 and caspase-8 activity, and reduced apoptosis as compared with B cells that have intact AR signaling.[168]

AR signaling is B cells is implicated in sex differences in humoral immune responses. Males often have lower antibody responses and reduced efficiency of B cells in germinal centers from male as compared with female mice contributes. Antigen-specific B cells are more likely to be positioned in the center of follicles found in secondary lymphoid tissues where germinal centers are formed. Increased expression of GPR174 (i.e., X chromosome GPCR) in germinal center B cells from males, which is the receptor for CCL21, induces migration of germinal center B cells to the T-B cell border of the follicles rather than toward the center.[169] Gonadectomy of male mice reduces, whereas testosterone treatment restores, GPR174-mediated B cell migration in males.[169] While this finding is not direct evidence of AR signaling in B cells, it suggests a role for AR signaling as a mediator of these intrinsic sex differences in germinal center B cells. In the absence of AR signaling in mice, serum concentrations of IgG3 and IgG2a as well as dsDNA IgG autoantibodies are increased compared with mice that have sufficient AR signaling. In the absence of AR signaling, specifically in B cells, titers of IgG-rheumatoid factor autoantibodies and

development of collagen-induced arthritis is increased.[170–172] These diverse murine models highlight that AR signaling is necessary for suppressing B cell activity, which has functional implications for autoimmunity.

## PROGESTERONE AND PR SIGNALING

Progesterone (produced by the corpus luteum) concentrations in serum vary across the life course between males and females, with increased fluctuations during puberty followed by cyclic changes during the menstrual cycle (Figure 1). In addition to E2, P4 concentrations change throughout life course and during development but are consistently greater in females compared with males. During pregnancy, P4 rises steadily and is sustained at high levels throughout gestation by the placenta in humans, followed by a decline post-partum, highlighting that hormone concentrations are altered across lifespan (Figure 1). There are several synthetic analogs of naturally occurring progesterone referred to as progestins that are used for contraception and replacement therapies.

PRs are found across diverse non-reproductive tissues, including lymphoid, gut, and brain. Progesterone regulates cellular functions through (1) direct nuclear "genomic" pathways that affect gene expression and transcription, or (2) indirect "nongenomic" membrane and cell-surface-bound progesterone receptors. Progesterone-activated, nuclear PRs (nPR) are highly expressed across diverse immune cells. There are functionally district nuclear receptor PR isoforms, PR-A, and PR-B, that bind to P4 and translocate to the nucleus to bind with progesterone response elements affecting downstream signaling and transcription. Although both isoforms are transcribed from PGR gene, these are functionally distinct receptors with the PR-A:PR-B ratio determining the impact of P4 on cellular transcriptional activity.[173] The other non-classical indirect progesterone pathway is membrane-bound PRs (mPR), which interact and activate mitogen-activated protein kinases (MAPKs) to affect gene transcription,[174] NF-κB by inhibiting gene transcription of cyclooxygenase-2 (COX-2) that results in decreased inflammation,[175] or through membrane-bound PRs increasing CAMP in the MAPK pathway.

Progesterone is generally inhibitory for immune cell function and mouse models highlight that the inhibitory effects occur through suppression of NF-κB activation[176] resulting in reduced COX-2 enzymatic activity[177] and synthesis of proinflammatory cytokines, including TNF and IL-1β,[178] as well as IL-12[179] in innate immune cells. In addition, pregnancy-associated changes in P4 and PR signaling results in immunological changes that are important for innate immune surveillance and tolerogenic responses.[180]

## PR SIGNALING IN INNATE IMMUNE CELLS

PR signaling generally inhibits innate immune cell function, with most studies focused on responses during pregnancy (e.g., when PR activity is elevated). The absence of PR signaling in mice reduces the number of circulating peripheral neutrophils.[181] Peripheral blood NK cells express PR[182] and NK cells isolated from humans are highly susceptible to P4-induced caspase-dependent cell death that can be reversed by treatment of NK cells with the anti-progestins, RU-486 and ZN98.299.[182] Uterine NK (uNK) cells are a unique population that comprise up to 70% of leukocytes in decidual tissues are involved in regulating placental development and have poor cytotoxic capacity.[183] Uterine NK cells do not express PR in either humans or mice.[184,185] Although P4 does not directly act on NK cells, it can regulate NK cell activity through glucocorticoid receptor (GR)-mediated reduced phosphorylation of STAT4 and IκB.[186] Progesterone also stimulates stromal endometrial cell production of IL-15 *in vitro*, which enhances proliferation of uNK cells.[187]

Progesterone inhibits inflammatory responses of macrophages and DCs, either directly through PR[188] or indirectly through altered pattern recognition receptor activity and cellular synthesis of cytokines. Progesterone reduces the production of NO and IL-12 in alveolar macrophages and bone-marrow-derived monocytes following LPS stimulation.[189] Following LPS exposure, P4 and dexamethasone (i.e., GR agonist), but not norgestrel (i.e., PR agonist), reduce NO synthesis in macrophages.[179] Progesterone treatment inhibits TLR4 and TLR9 activation, resulting in greater IL-6 and NO secretion from macrophages as well as impaired NF-κB activation.[176] PR signaling in DCs also inhibits TLR3- and TLR4-mediated production of IL-6 and the expression of costimulatory molecules.[190] In addition to downregulating costimulatory molecules, PR signaling inhibits DC expansion.[191] PR also functions through GR signaling to induce MAPK phosphatase in primary human myometrial cells.[192] Although limited data are available, P4 either signals through PR or GR to inhibit innate immune cell activity.

## PR SIGNALING IN T CELLS

Both nuclear and membrane PRs are expressed in human and murine T cells.[65,193–195] In T cells from the blood of healthy human females, mPR expression varies across the menstrual cycle.[196] mPR expression is 2-fold to 5-fold higher in $CD8^+$ T cells isolated during in the luteal as compared with the follicular phase of the menstrual cycle.[196] PR signaling generally suppresses activity in both $CD4^+$ and $CD8^+$ T cells. In human and mouse T cells, PR signaling diminishes $CD4^+$ and $CD8^+$ proliferation and activation and can cause apoptosis.[193,194,197]

The inhibitory effects of PR signaling in T cells are most pronounced during pregnancy, where PR activity prevents rejections of the semi-allogenic fetus, in part by promoting Treg activity.[198–201] In mice, blocking the PR with the antagonist mifepristone during pregnancy decreased the number of Tregs in the peripheral blood.[198] In pregnant human females, there is increased expression of PR-A in peripheral Tregs during the second and third trimester of pregnancy as compared with the day of delivery.[199,200] At delivery, PR-A+ Tregs are precipitously reduced, which may contribute to induction of contractions.[199] In $CD8^+$ T cells from pregnant mice, PR signaling promotes heme oxygenase 1 expression, leading to the development of tolerogenic $CD122^+$ $CD8^+$ T cells that promote fetal growth and vascularization.[202] PR signaling in γδ T cells plays a role during pregnancy. In mice, treatment of uterine-derived γδ T cells with P4 promoted expansion, whereas the same treatment of lung-derived γδ T cells represses.[203] PR signaling in γδ T cells

from pregnant human females with a history of spontaneous abortion yields fewer γδ T cells in peripheral blood and decidual tissue than from otherwise healthy pregnant females, with a positive correlation between γδ T cell number and expression of PR.[204] Limited data reveal that PR signaling in T cells is necessary to support a healthy pregnancy and fetal development.

## PR SIGNALING IN B CELLS

In both humans and mice, B cells express PR, with a predominance of PR-A.[103,205,206] During pregnancy, the number of pre-activated marginal zone B (MZB) cells is increased.[207] In mouse MZB cells stimulated LPS and CD40 and treated with P4, synthesis of IL-10, and to a lesser extent TNF, is reduced.[103] From healthy blood donors, Staphylococcal enterotoxin-B-activated PBMC cultures treated with P4 have reduced proportions of plasmablasts but increased frequencies of plasma cells.[208] From the limited data available, PR signaling alters B cell function, but how and whether this alters antibody production and transfer to offspring requires consideration.

## DISCUSSION

Sex steroid signaling impacts both innate and adaptive immune cell responses. To date, more studies have manipulated sex steroid ligands rather than receptors (Table 1). These studies clearly highlight diverse effects of sex steroid concentrations on immune function. We identify the molecular and cellular mechanisms altered by the binding of E2, P4, and testosterone to their respective receptors in immune cells, which could provide evidence for targetable therapies.

As nuclear receptors, sex steroid receptors directly regulate immune responses at the transcriptional level to alter disease outcomes. Studies in cancer immunology have made the most progress identifying the immunological pathways in innate and adaptive immune cells that are regulated by sex steroid receptor activation and have started to harness this for therapeutic treatments for reproductive cancers (e.g., breast and prostate cancers).[223,224] Application of findings in cancer immunology, including observations of AR signaling suppression of $CD8^+$ T cell function in the tumor microenvironment,[157,159] to other diseases, such as autoimmunity, inflammation, and immunity to infectious disease, that have sex-specific and sex-differential outcomes is needed. There also is a need for greater consideration of PR signaling within diverse immune cell populations, including DCs, macrophages, and NK cells, especially given the known immunological changes associated with morbidity and mortality from infections during pregnancy.[225–227]

Identification and improved specificity of the tools available for manipulating sex steroid concentrations and receptor activity both in animal models and humans will allow for deeper interrogation into how sex steroid signaling alters immune cell function. One of the most well-utilized models to study the cause and effect of sex steroids on immune function is removal of gonadal tissues (i.e., testes in males and ovaries in females) followed with exogenous replacement of the ligand (Table 1). There are, however, additional methodologies for manipulating sex steroid receptor activity in immune cells using knockout technologies and pharmacological receptor agonists and antagonists (Table 2). Global knockout of sex steroid receptors has been used to evaluate function of receptor activity.[68,69,164] Sex steroid receptor activity in specific immune cell types can be analyzed with adoptive transfer of cells from a global receptor knockout mice into immune cell depleted wild-type mice[155] or with a cre-loxP-mediated recombination system to knock out sex steroid receptors in a specific immune cell population.[228] For example, the select knockout of ERα in $CD4^+$ T cells[67,72] or *lysm* expressing macrophages[49] has yielded critical information about how ER signaling regulates the activity of these immune cells. The use of pharmacological sex steroid receptor agonists and antagonists (e.g., flutamide for AR, select ER modulators [SERMs] for ER, or mifepristone for PR[159,197,229]) has translational value for use in humans.

In preclinical and clinical settings, there can be bidirectional interactions between sex steroids and disease states, such that certain diseases can alter sex steroid concentrations and impact downstream signaling in immune cells. For example, hypogonadism (in both males and females) is a feature of several infectious diseases, including but not limited to HIV and tuberculosis, in humans.[233,234] In adult mice infected with influenza A viruses, suppression of androgens in males and disruption of the estrous cycle in females is a consequence of acute disease.[235,236] Detailed elsewhere are the diverse interactions between commensal microbes in the gastrointestinal tract and synthesis and metabolism of sex steroid, which alters immunity and disease outcomes often differentially between the sexes.[237,238] Commensal microorganisms can produce sex steroids, including androgens, to influence the immune landscape and disease outcomes.[239] In addition to microbes having bidirectional interactions with sex steroids, hormone-based treatments for reproductive cancers can alter immune responses. Breast cancer patients receiving the ER antagonist tamoxifen or prostate cancer patients receiving androgen deprivation therapy can have altered immune function.[152,240] With these phenotypic differences in immunological outcomes, comes a recognition that sex steroid receptor signaling transcriptional regulates the activity in immune cells, which could have novel therapeutic potential.

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## AUTHOR CONTRIBUTIONS

J.P.H., J.A.L., K.S., and S.L.K. all contributed to the generation of text, figures, and tables. All authors generated text edits, with S.L.K. acting as the lead editor.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

**Table 1. Effects of sex hormones on immune cell function**

| Immune cells | Estrogens | Androgens | Progestins |
|---|---|---|---|
| Innate | | | |
| Macrophage/microglia | promotes anti-inflammatory response through cytokine production, chemotaxis, and phagocytosis[37]<br>inhibits NF-κB pathway[38]<br>reduced TLR4 expression[39,40]<br>inhibits proinflammatory cytokine production[40] | enhances macrophage migration and anti-inflammatory cytokine production[41] | inhibits anti-inflammatory response[42]<br>inhibit TLR4 and TLR9 activation, and impair NF-κB activation[43,44] |
| Dendritic cells | promotes cell differentiation[58,209,210]<br>promotes proinflammatory cytokine production[210–212]<br>reduces IL-23 production[211]<br>enhances T cell stimulation[210,212] | decreases T cell stimulation[213]<br>decreased secretion of proinflammatory cytokines[214] | decreases secretion of proinflammatory cytokines[178] |
| Neutrophils | enhances neutrophil activation[45]<br>enhances cytokine and chemokine production[46]<br>enhances cell survival[47] Enhances NO synthase (nNOS) response[48] | regulate circulating neutrophil counts[49]<br>inhibits proinflammatory cytokine production[50]<br>promotes anti-inflammatory phenotypes and neutrophil accumulation impairing function[51,52] | |
| Natural killer cells | enhances NK cell number<br>inhibits NK infiltration, cytotoxicity, and proliferative capacity[53–55] | inhibits NK cell cytotoxicity and function[55–57] | enhances NK cell numbers 57 |
| Adaptive | | | |
| T cells | enhances Th17 responses[69,84,162,215,216]<br>enhances Th1 responses[217–219] | inhibits Th17 responses[84,162]<br>inhibits Th2 responses and memory[163]<br>inhibits Th1 responses[161]<br>enhances Treg responses[220]<br>inhibits lymphopoiesis[146,148,221] | inhibits $CD4^+$ and $CD8^+$ proliferation[194]<br>enhances Treg responses[198,201]<br>inhibits Th1 responses[222]<br>enhances Th2 responses[222]<br>inhibits Th17 responses[201] |
| B cells | enhances proliferation, differentiation, activation, and apoptosis[19–24]<br>enhances somatic hypermutation and class-switch recombination[25]<br>increase antibody production[22,25–30] | AR signaling KO increases frequency of B cells[31]<br>AR signaling KO increases antibody production[32–34] | increases frequency of marginal zone B cells during pregnancy[35]<br>decreases proportions of plasmablasts[36]<br>increases frequencies of plasma cells[36] |

AR, androgen; KO, knock out

**Table 2. Methods to evaluate sex steroid receptor function**

| Techniques to evaluate sex steroid receptor function | | | |
|---|---|---|---|
| Immune cells | Estrogen receptors | Androgen receptors | Progesterone receptors |
| **Innate** | | | |
| Macrophage/microglia | • 17β-estradiol (agonist)[44,54]<br>• selective ERα KO in macrophages[33,49]<br>• selective ERβ KO in macrophages[49] | • dihydrotestosterone (agonist)[144]<br>• selective AR KO in macrophages[141,144] | • norgestrel (agonist)[176,179] |
| Dendritic cells | • 17β-estradiol (agonist)[56]<br>• ICI 182,780 (antagonist)[60]<br>• global ERα KO[56,59–61]<br>• global ERβ KO[59,60]<br>• selective ERα KO in dendritic cells[56] | • defective AR mutant[230] | • norgestrel (agonist)[190]<br>• RU-486 (antagonist)[191] |
| Neutrophils | • PPT and DPN (agonist)[25]<br>• tamoxifen (antagonist)[23,26,30] | • dihydrotestosterone (agonist)[130]<br>• flutamide (antagonist)[131,132]<br>• global AR KO[128,132] | • PR KO[181] |
| NK cells | • 17β-estradiol (agonist)[36]<br>• tamoxifen (antagonist)[37]<br>•global ERα KO[33] | • enzalutamide (antagonist)[139]<br>• global AR knockdown[139] | • RU-486 and ZN98.299 (antagonist)[182] |
| **Adaptive** | | | |
| T cells | • PPT, 17β-estradiol, Zearalenone, DPN (agonist)[54,76,81,83]<br>• methyl-piperidino-pyrazole (MPP), OSU-Erb-012, ICI 182,780, raloxifene, ERB041 (antagonist)[70,76,77,83,91,229]<br>• global ERα KO[68,69,74,164,229,231]<br>• global ERβ KO[68,69,82,231]<br>• selective ERα KO in $CD4^+$ cells[67,71–73,75,163]<br>• selective ERβ KO in $CD4^+$ cells[163] | • degarelix, enzalutamide, flutamide (antagonist)[154,159]<br>• global AR KO[146,147,155,156,160,164]<br>• selective KO in $CD8^+$ cells[153]<br>• selective KO in $CD4^+$ cells[156,163]<br>• selective KO in FoxP3+ cells[160] | • mifepristone (antagonist)[197,198]<br>• selective KO in Lck+ cells[193]<br>• PR hemizygous mice[202]<br>• intracellular PR KO[232] |
| B cells | • 2-Methoxyestradiol, 17β-estradiol (agonist)[97–100]<br>• tamoxifen, fulvestrant (antagonist)[100,102]<br>• global ERα KO[108] | • dihydrotestosterone (agonist)[168]<br>• global AR KO[168,170–172] | • progesterone (agonist)[103,208] |

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