Sex, Cells, and Asthma

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Abstract

There are marked sex differences in asthma prevalence and severity. Sex hormones play a central role in these sex biases and directly interact with multiple key cells involved in the pathogenesis of asthma. Here we review the known effects of estrogen, progesterone, and testosterone on airway epithelial cells, airway smooth muscle cells, the mononuclear phagocyte system, innate lymphoid cells, eosinophils, mast cells, T cells, and B cells, all in the context of asthma. Furthermore, we explore unresolved clinical questions, such as the role of sex hormones in the link between asthma and obesity.

Sex differences in the lung have long been recognized at all life stages, from embryogenesis to adulthood.1-3 Such differences are understood to be important in contributing to lung development, growth, and adaptations to environmental stimuli. Notably, the modulatory effects of sex steroids (estrogens, progesterone, and testosterone) are manifested in outcomes of premature birth between male and female babies, the impact of key life points (such as puberty, postpubertal growth, and menopause), and aging. Furthermore, these sex differences are increasingly recognized as

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**Learning Objectives:**

1. Identify the mechanisms by which sex hormones regulate different cell types involved in the pathogenesis of asthma.
2. Contrast the effects of estrogen, progesterone, and testosterone on immune cells and resident airway cells.
3. Describe future directions in the field, including the intersections of sex steroid signaling with innervation or metabolism.

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relevant in pulmonary disease pathogenesis and outcomes, especially in asthma.

A multitude of human studies have reported the impact of sex, puberty, menstruation, pregnancy, and menopause as well as of aging on the incidence and severity of asthma, suggesting roles for intrinsic differences as well as sex steroids in modulating airway disease. Conversely, oral contraceptive drug (OCD) use worsens asthma symptoms, and hormone replacement therapy (HRT) is associated with an increased risk of new-onset asthma. The influence of male sex hormones in the pathophysiologic process of asthma is suggested by reports that lower levels of testosterone correlate with a lower forced expiratory volume in 1 second, data from our group that elevated testosterone is associated with decreased asthma, and finally findings that nebulized dehydroepiandrosterone 3-sulfate improves asthma control. In spite of these clinical studies, the cellular mechanisms by which sex hormones regulate the pathophysiologic process of asthma are not fully understood. In this article, we discuss the effects of sex hormones in the various cell types potentially involved in the pathogenesis of asthma (Figure). Here we stress the importance of both immune cells and resident airway cells in mediating and modulating sex steroid effects. The article does not focus on the role of intrinsic sex differences in the lung, and the reader is referred to several excellent articles on this important topic.

**SEX STEROID RECEPTORS AND SIGNALING**

The effects of estrogen are largely mediated by two distinct estrogen receptors (ERs), ERα and ERβ. Both of these receptors belong to the nuclear receptor family of transcription factors and bind to the same DNA response elements. The two ERs are

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**FIGURE.** Summary of the key effects of estrogen, progesterone, and testosterone on structural and immune cells relevant to the pathogenesis of asthma. Also illustrated are the male-female cell-specific differences in asthma. AAM, alternatively activated macrophage; AChE, acetylcholinesterase; AHR, airway hyperreactivity; DC, dendritic cell; IgE, immunoglobulin E; IL-4, interleukin 4; ILC2, type 2 innate lymphoid cell; LN, lymph node; MCP-1, monocyte chemoattractant protein 1; NO, nitric oxide; OVA, ovalbumin; SOCE, store-operated calcium entry; STIM1, stromal interaction molecule 1.
homologous in their ligand-binding domain but significantly differ in their amino-terminal domains.42 Pathways targeted by ligand activation of ERs include direct DNA binding to estrogen response elements located in the promoters of target genes, protein-protein interactions with other DNA-binding transcription factors, and non-genomic effects.43 Interestingly, ERβ can function as an inhibitor of ERα-mediated transcriptional activity and decrease the cellular response to estrogen.44,45 In addition to these “classical” signaling aspects, there is now evidence for both ERs on cellular plasma membranes and within the cytoplasm, with nongenomic and genomic signaling through pathways such as mitogen-associated protein kinases, phosphatidylinositol kinase, and Akt relevant to inflammation and through cellular proliferation and other aspects that could contribute to lung health and disease.46 Furthermore, in several cell types, there is evidence for a G protein–coupled ER (also termed GPR30)46 that may be the basis for nongenomic signaling. However, there is limited or no information on a G protein–coupled ER in asthma.

Progesterone effects are mediated by 2 isoforms of progesterone receptors (PRs), PR-A and PR-B. In humans, both isoforms are transcribed by the same gene but differ in that the A isoform has a truncated amino-terminal domain.47 Activation of PRs typically leads to the interaction with progesterone response elements and the downstream regulation of target genes, with PR-B functioning as a transcriptional activator and PR-A as a repressor of gene transcription.47 In addition, PRs can also directly interact with regulatory proteins and signal through nongenomic pathways.48

Finally, both testosterone and its highly active metabolite 5α-dihydrotestosterone act through the androgen receptor, also known as NR3C4. As with the other sex steroid receptors, activation of the androgen receptor leads to binding to androgen response elements and downstream up-regulation or down-regulation of target genes.50

AIRWAY EPITHELIAL CELLS

Airway epithelial cells are essential controllers of the initiation and progression of allergic and inflammatory diseases, such as rhinosinusitis (nasal epithelium) and asthma (bronchial epithelium). Airway epithelial cells express pattern recognition receptors that can sense a vast array of environmental stimuli, including allergens, microbes, and particulate matter air pollution. In response to these environmental stimuli, airway epithelial cells release several endogenous danger signals, such as uric acid, adenosine triphosphate, lysophosphatidic acid, thymic stromal lymphopoietin, interleukin (IL) 25, granulocyte-macrophage colony-stimulating factor, and IL-33 and other members of the IL-1 family. These endogenous danger signals orchestrate the activation of dendritic cells (DCs) as well as of innate lymphoid cells (ILCs), therefore acting as a pivotal bridge between the innate and adaptive immune systems and ultimately controlling allergic inflammation.51,52

Nasal Epithelial Cells

The messenger RNA (mRNA) of ERα and ERβ has been detected in human nasal mucosa.53 Furthermore, multiple studies have found that sex hormones can directly affect the nasal mucosa. For instance, Ellegard and Karlsson54 found that the degree of nasal congestion fluctuates during a healthy woman’s menstrual cycle. In their study, morning nasal peak expiratory flow measurements were the lowest during menstrual days, when serum estrogen levels reach their nadir. Others have reported that during ovulation, when estrogen levels peak, the nasal mucosa of healthy fertile women becomes hyperreactive to histamine. This hyperreactivity is not observed during the menstrual or luteal phases.55

Further evidence to suggest a direct effect of sex hormones on the nasal mucosa comes from nasal provocation tests with an OCD. One study found that more than 80% of women who recalled having nasal complaints with an OCD had a positive nasal mucosa response after topical challenge with the same drug. These responses were
attributed to the estrogen component of the OCD and were consistent with type I (or immediate) hypersensitivity reactions. Others have reported that both estrogen and progesterone can significantly increase the levels of histamine receptor H1 mRNA in human nasal epithelial cells. Such sex hormone–induced up-regulation of histamine receptor H1 might partially explain why some women experience worsening of their nasal symptoms during pregnancy. Finally, studies in pregnant women have also contributed to our understanding of the effects of sex hormones on the nasal mucosa. As an example, the nasal mucosa of pregnant women, regardless of the duration of pregnancy, has increased glandular hyperactivity, a more robust phagocytic activity, and higher levels of acid mucopolysaccharides.

**Bronchial Epithelial Cells**

Normal human bronchial epithelial cells (BECs) express twice as much ERβ as ERα, with both receptors located in the cytoplasm, nucleus, and mitochondria. Such receptors are likely to be functional on the basis of data that estradiol can induce an estrogen response element–mediated reporter luciferase activity in transiently transfected human BECs, interestingly from both men and women. However, their downstream signaling pathways, particularly in the context of disease, have not been extensively examined.

Estrogen can regulate epithelial calcium signaling, which can have downstream influences on other cellular processes. For example, Sheridan et al have reported that estrogen can inhibit the translocation of stromal interaction molecule 1 in airway epithelial cells through serine phosphorylation of the stromal interaction molecule protein, thus preventing store-operated calcium entry. Such changes in calcium homeostasis can result in downstream effects on ion transport, growth, and cell division in the airway epithelial barrier.

Nitric oxide (NO) is a potent endogenous bronchodilator capable of regulating airway tone in both physiologic and pathologic states. In menstruating women, exhaled NO concentrations are highest during ovulation, which coincides with the peak in circulating estrogen concentrations. Our group has found that estradiol and ER isoform–specific agonists can stimulate NO production from BECs, whereas ER antagonists have an inhibitory effect. The effects of estrogen on NO production can be mediated by caveolin 1 and by an increase in intracellular calcium concentration. In addition, estrogen can increase endothelial NO synthase activation and contribute to bronchodilation. Similarly, another group found that estrogen, through ERα, can trigger the influx of extracellular calcium and elicit a rapid increase in endothelial NO synthase activity in human BECs. Overall, these studies suggest that estrogen-induced NO production from BECs can have a significant impact on airway tone. Whether such effects are relevant in both sexes is unclear, but given the possibility of aromatase-mediated conversion of testosterone to estradiol, sex steroid effects on BEC-derived NO need to be further explored.

The airway epithelial barrier of patients with asthma is characterized by an increase of basal and goblet cells, a decrease of terminally differentiated ciliated cells, and an overall loss of barrier integrity with epithelial to mesenchymal transition. We have observed that treatment of normal human BECs grown in an air-liquid interface with estradiol can further promote this epithelial to mesenchymal transition and loss of barrier integrity (Stelzig K, Prakash YS, Chiarella SE, unpublished observations, February 2021). Estrogen can also exert effects on airway epithelial mucus production. Normal human BECs differentiated in an air-liquid interface and treated with physiologic doses of estrogen have higher numbers of MUC5AC-positive (goblet) cells compared with the vehicle-treated cells. These effects are attenuated by an ERβ antagonist. Estrogen effects on mucus production appear to involve inflammatory pathways such as nuclear factor of activated T cells, cytoplasmic 1 in ERβ effects on MUC5AC mRNA and protein and extracellular signal-regulated kinase 1/2—mitogen-activated protein kinase.
in ERα-induced increases in MUC5B expression.67

Finally, although less studied, progesterone levels can also have an impact on the mucociliary apparatus of the airway epithelium. For example, Jain et al68 have reported that PRs are expressed in proximal ciliated airway epithelial cells (isoform PR-B being more abundant than PR-A). Furthermore, treatment of airway epithelial cells with progesterone leads to a significant decrease in ciliary beat frequency. Interestingly, this effect is time and dose dependent and is inhibited by the co-administration of estradiol with progesterone.68

In summary, sex steroids are able to regulate multiple aspects of airway epithelial barrier function. Estrogen has been reported to regulate calcium and downstream NO production (largely through ERα), mucus production (largely through ERβ), and expression of histamine receptors. In addition, progesterone has been found to inhibit the mucociliary apparatus.

AIRWAY SMOOTH MUSCLE CELLS
Airway smooth muscle (ASM) cells are involved not only in airway tone and airway hyperresponsiveness (AHR) but also in inflammatory and remodeling processes that contribute to airway disease. For instance, ASM cells produce and influence the deposition of extracellular matrix proteins and regulate the local inflammatory milieu through the production of cytokines, chemokines, and growth factors.69 Here we review the effects of sex hormones on ASM cells and AHR.

Degano et al70 reported that oophorectomized female rats treated with physiologic doses of estrogen have significantly less AHR to acetylcholine compared with placebo-treated rats. Similarly, in measuring ex vivo AHR, estrogen treatment of isolated tracheal segments increases the concentration of acetylcholine required to achieve a predetermined contractile force. Interestingly, these differences are abolished by removing the epithelium. Furthermore, acetylcholinesterase activity is higher in estrogen-treated tracheas. These results indicate that estrogen decreases AHR through epithelium-dependent increases in acetylcholinesterase activity and probably involves ASM.70 Similarly, others have found that estrogen can potentiate isoproterenol-, epinephrine-, and norepinephrine-induced relaxation of the pig bronchus.71 Overall, these studies highlight the role of estrogen in regulating AHR and the potential role of ASM in the effects of sex hormones.

There are different mechanisms by which estrogen can regulate ASM cell function. For instance, estrogen can activate the NO—cyclic guanosine monophosphate—protein kinase G pathway to promote calcium-activated potassium channel activity, thus causing membrane hyperpolarization, an effect observed in estrogen inhibition of cholinergic constriction of asthmatic tracheal rings.72 Our group has found that ASM cells express both ER isoforms, with both nuclear and nonnuclear localization such that acute treatment of human ASM cells with physiologic doses of estrogen reduces the intracellular calcium responses to histamine. These effects are mediated by a decrease in calcium influx through L-type channels. Interestingly, the reduction of intracellular calcium is more pronounced with an ERα-selective agonist than with an ERβ-selective agonist.73

The ability of estrogen to regulate AHR has also been observed in a murine model of ovalbumin (OVA)—induced allergic inflammation,74 but interestingly, estrogen is seen as protective. For example, oophorectomy or the administration of an estrogen antagonist in female mice results in enhanced AHR. Conversely, estrogen exerts the opposite effect in male mice, suppressing AHR in a dose-dependent manner. On the basis of additional data from experiments using a neurokinin 1 receptor antagonist, estrogen effects on AHR are thought to involve neurokinin 1—prejunctional activation of ASM cells.74

The effects of progesterone have also been examined to some extent. For example, in a murine ozone model, progesterone reduces airway remodeling and improves glucocorticoid resistance.75 In the pig bronchus, progesterone, albeit at supraphysiologic concentrations, potentiates isoproterenol-
induced bronchodilation. However, what is not known is the interaction between estrogens and progesterone, given their usual concurrent presence in vivo, in terms of airway contractility, relaxation, or other more chronic effects.

The data regarding the effects of testosterone on ASM cells and AHR are less consistent, with different opposite effects reported in the literature. For instance, testosterone has been reported to potentiate isoproterenol-induced bronchodilation of the pig bronchus. In addition, Espinoza et al have reported that dehydroepiandrosterone is able to induce relaxation of ASM in an epithelium- and NO-independent mechanism that involves the blockage of voltage-dependent calcium channels. In contrast, there is also evidence that testosterone can promote AHR. Castration of male mice leads to a decrease in AHR, which is partially restored by the administration of exogenous testosterone. The restoration of AHR by exogenous testosterone in castrated male mice or female mice is abolished by bilateral vagotomy. Thus, testosterone may promote cholinergic AHR by regulating vagally mediated reflex mechanisms. Several studies highlight the role of macrophages in regulating sex differences in asthma. In the OVA model of allergic inflammation, female lungs have greater numbers of alternatively activated macrophages (AAMs) compared with male lungs. Such increased numbers of AAMs are thought to result in an increased migration of inflammatory myeloid DCs from the lungs to the lymph nodes, more robust T-cell expansion, and worse downstream airway inflammation. Importantly, male and female mice have comparable numbers of regulatory T cells in the lung and similar regulatory T-cell function. In a subsequent study using a house dust mite-induced model of allergic inflammation, the same group confirmed that female mice have higher numbers of CD68-positive cells (total macrophages) and YM1-positive cells (M2 macrophages) in lung tissue compared with male mice. Furthermore, the numbers of M2 macrophages in lung tissue positively correlate with the percentage of eosinophils in the bronchoalveolar lavage (BAL) fluid of house dust mite–exposed mice. There are no sex differences in interferon regulatory factor 5–positive (M1 macrophages) and IL-10–positive (M2-like macrophages) cells in lung tissue. In contrast to these data regarding the promotion of acute airway allergic inflammation by AAMs, there is evidence to suggest that AAMs can be

**THE MONONUCLEAR PHAGOCYTE SYSTEM**

The mononuclear phagocyte system of the lung is composed of monocytes, macrophages, and DCs. All these cell types have been found to have pivotal roles in asthma pathogenesis.

**Monocytes and Macrophages**

Sex hormones can have opposing effects in different phases of monocyte recruitment. On the one hand, estradiol and progesterone can stimulate monocyte chemoattractant protein 1 mRNA and protein expression from endothelial cells, thus promoting the recruitment of monocytes. On the other hand, estradiol and raloxifene (a selective ER modulator) have been reported to inhibit monocyte chemoattractant protein 1–induced monocyte migration by engaging ERα but not ERβ.

Several studies highlight the role of macrophages in regulating sex differences in asthma. In the OVA model of allergic inflammation, female lungs have greater numbers of alternatively activated macrophages (AAMs) compared with male lungs. Such increased numbers of AAMs are thought to result in an increased migration of inflammatory myeloid DCs from the lungs to the lymph nodes, more robust T-cell expansion, and worse downstream airway inflammation. Importantly, male and female mice have comparable numbers of regulatory T cells in the lung and similar regulatory T-cell function. In a subsequent study using a house dust mite–induced model of allergic inflammation, the same group confirmed that female mice have higher numbers of CD68-positive cells (total macrophages) and YM1-positive cells (M2 macrophages) in lung tissue compared with male mice. Furthermore, the numbers of M2 macrophages in lung tissue positively correlate with the percentage of eosinophils in the bronchoalveolar lavage (BAL) fluid of house dust mite–exposed mice. There are no sex differences in interferon regulatory factor 5–positive (M1 macrophages) and IL-10–positive (M2-like macrophages) cells in lung tissue. In contrast to these data regarding the promotion of acute airway allergic inflammation by AAMs, there is evidence to suggest that AAMs can be
important in promoting tissue repair. Here, estradiol and ER-selective agonists have been reported to induce alternative macrophage activation through ERα, positively contributing to wound repair.83

**Dendritic Cells**

Dendritic cells are the most potent antigen-presenting cells and play an essential role in inducing allergen-specific inflammatory responses. Allergens can directly activate DCs through pathogen-associated molecular patterns or indirectly by engaging airway epithelial cells. Importantly, DCs not only promote allergic inflammation, but certain DC subsets are involved in the development of tolerance to allergens.84

Several groups have found that estrogen can regulate tissue-dependent DC differentiation, maturation, and cytokine production. For instance, Paharkova-Vatchkova et al85 have reported that estrogen but not testosterone is required for ex vivo DC differentiation and for the ability of DCs to present antigens and to stimulate the proliferation of naïve CD4+ T cells. Specifically, estrogen is important for the induction of a CD11c+, CD11bintermediate DC population with potent antigen-presenting capacity. Furthermore, DC differentiation can be inhibited by ER antagonists and by genetically deleting ERα from bone marrow cells.

In a murine model of OVA-induced allergic lung inflammation, female mice have higher numbers of CD11bhigh DCs and CD103+ DCs in the lung and bronchial lymph node compared with male mice. CD103+ DCs from female mice also have enhanced antigen uptake ability. Finally, CD4+ T cells cultured with CD103+ DCs from female mice produce higher quantities of type 2 cytokines, such as IL-4, IL-5, and IL-13, compared with those cultured with CD103+ DCs from male mice.86

**INNATE LYMPHOID CELLS**

The ILCs, particularly group 2 ILCs (ILC2s), play a key role in type 2 inflammatory responses, such as eosinophilic asthma. ILC2s are activated by alarmins, such as IL-33, that are secreted by the airway epithelium. Activated ILC2s produce IL-5 and IL-13, which lead to airway eosinophilia and AHR.

Group 2 innate lymphoid progenitors express the androgen receptor.87 Androgen receptor signaling impairs the differentiation of these progenitors into mature ILC2s. Furthermore, male mice have a reduced expansion of lung ILC2s during allergic airway inflammation compared with female mice. Interestingly, orchietomy but not oophorectomy abolishes these sex differences in ILC2 differentiation and lung inflammation.87 Cephus et al87 also found that women with moderate to severe asthma have increased numbers of circulating ILC2s compared with men. In addition, the authors also reported that administration of 5α-dihydrotestosterone decreases the numbers of lung ILC2s and IL-5 and IL-13 expression from these cells. Finally, testosterone can decrease Alternaria-induced airway inflammation, including IL-5+ and IL-13+ ILC2s and lung eosinophils. Consistent with these findings, ex vivo IL-33 stimulation of ILC2s from female mice leads to an increase in the protein levels of IL-5 and IL-13. These findings are not seen in ILC2s from male mice.89

Most recently, Kadel et al90 reported that female mice have higher numbers of ILC2s at baseline, in part owing to higher numbers of killer-cell lectin-like receptor G1 (KLRG1)—negative ILC2s. KLRG1− ILC2s increase with age after sexual maturity and are capable of producing type 2 cytokines. Furthermore, androgens but not estrogens are able to regulate the numbers and function of these KLRG1− ILC2s.

Interestingly, Bartemes et al91 found that ILC2s are expressed in the mouse uterus and that these cells are responsive to IL-33. Furthermore, oophorectomy led to a decrease in uterine ILC2s, and estrogen treatment had the opposite effect. These changes were not observed in lung ILC2s, which suggests that the effects of estrogen on ILC2s are organ specific.

**EOSINOPHILS**

Eosinophils have multiple roles both in the normal lung and in asthma. Eosinophils not only are a key component of allergic
Airway inflammation but also play important functions in host defense and metabolism. Eosinophils express ERs, and β-estradiol can enhance the adhesion of eosinophils to endothelial cells. In addition, estradiol promotes degranulation of eosinophils, both in vivo and in vitro. Sex differences in airway and parenchymal eosinophil infiltration are consistent across a wide variety of murine models of allergic airway inflammation, with female mice having higher numbers of eosinophils compared with male mice. A role for estradiol in eosinophil infiltration is further supported by studies finding that both ERα and ERβ agonists can augment eosinophilic inflammation. Treatment of male mice with estradiol also results in increased blood and airway eosinophils. Conversely, estrogen antagonists suppress the mobilization of bone marrow eosinophils and their migration to the airways. However, the effect of estrogens is not always clear-cut. For example, Riffo-Vasquez et al proposed a dual role of estrogen in allergic airway inflammation. Oophorectomy of female mice before the sensitization phase in a mouse model of asthma leads to decreased eosinophils and IL-5 in lung lavage fluid. Surprisingly, if oophorectomy is performed after the sensitization phase, there is no change in lung eosinophilia but an increase in IL-5. This study suggests that estrogen might play differential roles in the sensitization and elicitation phases of the allergic response.

Testosterone can also regulate eosinophil biology. Testosterone-treated eosinophils have decreased adhesion to endothelial cells and viability. Furthermore, castrated male mice in a murine model of asthma have higher levels of eosinophils in BAL fluid compared with sham-operated mice. This suggests that testosterone attenuates the recruitment of eosinophils into the lung during allergic inflammation.

Mast cells play a prominent role in the initiation and progression of airway inflammation and AHR. Several groups have found the expression of estradiol and PRs in mast cells and outlined the ability of sex hormones to regulate mast cell biology. Specifically, multiple studies have reported that estradiol can regulate mast cell degranulation and promote IgE-mediated release of allergic mediators such as histamine and leukotriene C4. Interestingly, Zaitso et al reported that some of these estradiol effects occur through the engagement of a non-nuclear ERz and downstream influx of extracellular calcium. Furthermore, murine studies have found that mast cells from female mice, compared with those from male mice, exhibit an increased release of allergic mediators, and this difference is associated with an increased capacity for synthesis and storage of these granule-associated mediators. Progesterone treatment has been reported both to stimulate and to inhibit mast cell degranulation. Finally, mast cells also express the androgen receptor. Testosterone seems to have no effect or anti-inflammatory effect on mast cells.

T cells play pivotal roles in asthma, especially CD4+ T cells, and Th1, Th2, and Th17 lineages are important in distinct asthma phenotypes. ERα and ERβ are expressed in CD4+ T cells. Women have a higher number of blood CD3+ and CD4+ T lymphocytes compared with men. In an OVA-induced model of allergic inflammation, female mice have higher levels of CD4+ T cells compared with male mice. In addition, Maret et al reported that administration of estradiol to castrated female mice leads to a significant expansion of antigen-specific CD4+ T cells and promotes the development of interferon γ—producing (Th1) T cells. Furthermore, the authors reported that the expression of ERα but not of ERβ in hematopoietic cells is required for the effects of estradiol on these Th1 responses. Finally, it has also been reported that estradiol can induce IL-4 secretion and GATA-3 expression in CD4+ T cells and that these effects are abrogated by deleting ERα.
B CELLS

B cells and plasma cells play a key role in asthma pathogenesis. Plasma cells produce IgE antibodies that bind to the high-affinity FcεRI on mast cells and basophils. On antigen reexposure, these cells release mediators that contribute to immediate hypersensitivity reactions and bronchoconstriction. In addition, B cells also function as antigen-presenting cells in murine models of allergic airway inflammation.120

Women have higher percentages and absolute counts of blood B cells compared with men.121 B cells express ERα and ERβ.116,122 Estrogen treatment leads to the up-regulation of Bcl-2, which protects B cells from receptor-mediated apoptosis, resulting in increased survival and reduced B cell tolerance.122,123 Similarly, in murine models of allergic inflammation, female mice have higher levels of B cells99 and allergenspecific IgE97,99,100 compared with male mice.

Estradiol has been reported to promote the induction of regulatory B cells.124 In addition to promoting the survival of B cells, estrogen also enhances their immunoglobulin production. Han et al125 found that estradiol induces an increase in both IgM and IgE levels. Estradiol also causes a dose-dependent increase in the production of IgM and IgE human peripheral blood mononuclear cells, without altering cell viability and proliferation.

Interleukin-4 is an important cytokine for B cell differentiation, activation, and class switching to IgE. Lambert et al119 have reported that estradiol, through ERα, can increase IL-4 secretion and GATA-3 mRNA levels in CD4+ T cells. Other work suggests that increased levels of estradiol and progesterone correlate with increased IL-4 production in the luteal phase of the ovarian cycle.120 In addition, studies using models of allergic airway inflammation have found higher levels of IL-4 in the BAL fluid and whole lung,86,97,99,100 but the role of sex steroids has not been examined.

Androgen receptors are present in immature B cells,127 and androgens can have important suppressive effects on B-cell development. Castration of normal male mice results in a long-lived expansion of the bone marrow B-cell population and an increase in the numbers of mature B cells.127-129 These effects of castration are reversed by androgen replacement.128 In line with these findings, castrated male mice sensitized with phospholipase A2 (PLA2) have higher levels of PLA2-specific IgE compared with sham-operated mice. In addition, androgen treatment of the castrated mice leads to a reduction in PLA2-specific IgE production.130 Furthermore, castration also increases IL-4 levels in splenic cells, suggesting that androgen could also have an effect on IgE class switching.98 Finally, a similar effect has been reported in humans as androgens are able to suppress immunoglobulin production by directly inhibiting B-cell function.131

UNRESOLVED CLINICAL QUESTIONS AND FUTURE DIRECTIONS

There are several unresolved issues with regard to the effects and importance of sex hormones in the pathogenesis of asthma. First, the general aspects of sex steroid receptor expression and potential signaling pathways are highly cell type and context dependent, further modulated by whether receptors are nuclear or nonnuclear. Furthermore, in women, the concurrent presence of both estrogens and progesterone at varying levels, depending on life events such as menstrual cycle, pregnancy, and menopause, makes it likely that there are complex, little understood interactions between these hormones in any organ, and in this regard, the lung has been barely examined. Even in men, testosterone conversion to estradiol through aromatase can complicate the overall effect of sex steroids in any context. These considerations are important toward a comprehensive understanding of the influence of sex steroids in the context of asthma in cell-specific fashion.

Variations in asthma prevalence and severity during the perimenopausal and postmenopausal periods is a particularly active area of research. The perimenopausal period is often associated with a significant decline in lung function and worsening of asthma symptoms.28 Furthermore, the onset of menopause is also associated with an
increase in the frequency of asthma exacerbations. Interestingly, a meta-analysis by Zemp et al reported increased prevalence of asthma among postmenopausal women receiving HRT but not in those not taking HRT. The onset of asthma after menopause is frequently associated with a unique phenotype characterized by less atopy, increased eosinophilic airway inflammation, and frequent severe asthma exacerbations. Do fluctuations in sex hormone levels present during the perimenopausal period play a role in the emergence of this distinctive asthma phenotype? If so, which are the cell types involved in this asthma endotype? These are important questions that warrant further investigation.

Another interesting aspect of asthma potentially relevant to sex steroid levels and signaling is the clear interaction between sex and obesity. For instance, in a study using an OVA-induced murine model of allergic lung inflammation, female mice that were fed a high-fat diet had a higher accumulation of leukocytes in their lung parenchyma compared with their male counterparts. In addition, these female mice that were fed a high-fat diet developed more pronounced lung remodeling, higher deposition of transforming growth factor β, and lower levels of epithelial cell adhesion molecule compared with their lean female controls and male mice. There also seems to be an interaction between ASM cells and adipocytes. A study using primary human ASM cells and adipocyte explants found that ASM cells incubated with adipocyte-conditioned media released higher levels of IL-6 and eotaxin compared with ASM cells that were not conditioned. There were no differences in ASM cell migration, proliferation, or contractility between the groups. How sex steroid levels are involved in these contexts and the relevant role of estrogenic signaling remain to be established.

Many epidemiologic studies have found obesity to be a risk factor for asthma. This association seems to be modified by sex, with older obese women being most vulnerable to development of late-onset asthma. The mechanisms underlying the associations between sex hormones, obesity, and asthma are complex, considering there is substantial cross-regulation between sex hormones, adipokines, and inflammatory cytokines implicated in the pathogenesis of asthma.

Han et al noted that obese women but not obese men with the greatest levels of serum testosterone and independently also the greatest levels of serum estradiol had 41% and 56% reduced odds of current asthma relative to obese women with the lowest levels of these hormones, respectively. These results suggest that both male and female hormones can attenuate the proasthmatic stimulus of obesity among women. The mechanism for this attenuation is unclear, but it may relate to metabolic and inflammatory pathways associated with insulin resistance, which we have previously observed to be the metabolic syndrome component primarily responsible for magnifying the association between obesity and asthma. One of the ways that insulin resistance may cause asthma is through preferential insulin receptor hyposensitization in tissues other than the lung (liver, muscle, adipocytes), which would leave the lung susceptible to compensatory hyperinsulinemia. In turn, hyperinsulinemia can exert proasthmatic effects on the airway by inducing ASM hypercontractility, reductions in the endogenous bronchodilator NO, and other mechanisms.

Interestingly, supplementation of both estrogen and testosterone counteracts insulin resistance in tissue-specific fashion, which raises the possibility that sex hormones mitigate the increased risk of asthma seen in obesity by reducing insulin resistance. Why this effect preferentially occurs in obese women over obese men is unclear. If not through insulin resistance, reduced estrogen and testosterone availability may magnify the obesity-related risk of asthma through increases in IL-6, a cytokine that tends to correlate with insulin resistance. IL-6 is essential for allergen-induced mucus hypersecretion, a feature exhibited by many individuals with asthma. Elevated IL-6 levels associate with worse asthmatic features even in nonobese patients, suggesting a proasthmatic role for IL-6.
independent of obesity. Curiously, among female mice exposed to a high-fat diet, those that underwent ovariectomy had a 90% increase in IL-6 relative to those that underwent sham surgery. An analogous IL-6 increase is not seen in an obese mouse model of reduced androgen signaling. These findings suggest that estrogens ameliorate obesity-related increases in IL-6 levels in women. Whether testosterone also ameliorates IL-6 increases in obese women is unknown, but it does not seem to have this effect in men, which may align with the human clinical data reported by Han et al.

In multiple asthma phenotypes, there is increasing recognition that innervation plays a role in both airway irritability and AHR but also in chronic airway remodeling. The roles of sympathetic and particularly parasympathetic innervation in regulation of airway tone and reactivity are well recognized, as are roles in defensive reflexes of the upper airway, such as coughing and sneezing. Human airways are innervated by nonadrenergic, noncholinergic (NANC) pathways involving excitatory bronchoconstriction through tachykinins, substance P, and neurokinin A and bronchodilation, whereas inhibitory NANC pathways involve vasoactive intestinal peptide and NO. Thus, imbalances between excitatory and inhibitory NANC pathways can contribute to AHR. No specific information is available regarding how sex steroids influence NANC pathways and their potential contribution to sex differences in asthma. Peripheral neurons can also produce steroids and have the machinery for using cholesterol and thus producing sex steroids. Thus, there are multiple potential points of intersection between innervation, asthma, and sex differences that remain largely unexplored.

**CONCLUSION**

There is increasing evidence regarding the effects of sex steroids in the pathophysiologic process of asthma, resulting in sex differences that go beyond intrinsic structural or functional differences. Here, sex steroid metabolism, receptor expression, downstream signaling pathways, and interactions between steroids are all important, with temporal and contextual variations in their contributions. In this regard, sex steroid effects may be beneficial or detrimental, but it remains to be established whether and how such effects occur and their validity at different ages or during specific life events. Here, it may be important to consider circulating sex steroid levels as well as potential effects of local steroid metabolism, specific receptor expression/function and their localization, interactions between sex steroids and other modulatory factors such as inflammatory mediators and adipokines (e.g., in the context of obesity), and finally age. Overall, complex sex steroid signaling in the lung is clearly important and should be considered from both research and clinical perspectives, with the intent of developing novel therapeutic avenues on the basis of sex and gender in today’s world of individualized medicine.

**Abbreviations and Acronyms:**

- AAM = alternatively activated macrophage
- AHR = airway hyperresponsiveness
- ASM = airway smooth muscle
- BAL = bronchoalveolar lavage
- BEC = bronchial epithelial cell
- DC = dendritic cell
- ER = estrogen receptor
- HRT = hormone replacement therapy
- IL = interleukin
- ILC = innate lymphoid cell
- KLRG1 = killer-cell lectin-like receptor G1
- mRNA = messenger RNA
- NANC = nonadrenergic, noncholinergic
- NO = nitric oxide
- OVA = ovalbumin
- PLA2 = phospholipase A2
- PR = progesterone receptor

**Grant Support:** This work was supported by National Institutes of Health grants K08 AI141765 (S.E.C.), U54 AG044170 (S.E.C.), K23 AI125785 (J.C.C.), R01 HL142061 (Y.S.P.), R01 HL088029 (Y.S.P.), and R01 HL056470 (Y.S.P.).

**Potential Competing Interests:** Dr Cardet reports receiving honoraria from AstraZeneca for work in its Advisory Board on eosinophilic diseases, unrelated to this manuscript. Dr Chiarella received grant/research support from the National Institutes of Health (NIH)/NIH/National Institute of Allergy and Infectious Diseases (NIAID) (secondary investigators need not disclose). Dr Prakash reports no competing interests.

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