

Are There Reasons Why Adult Asthma Is More Common in Females?

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Many epidemiological studies suggest that women are at increased risk of developing adult-onset asthma and also suffer from more severe disease than men. These gender differences appear to be the product of biological sex differences as well as sociocultural and environmental differences. The biological sex differences include genetic, pulmonary, and immunological factors. There is compelling evidence that sex hormones are major determinants of at least these biological sex differences. This paper explores the current literature regarding effects of sex hormones on immune function, resident lung cells, and regulation of local processes in the lung to shed light on underlying mechanisms of gender differences in asthma. More research is needed to understand these mechanisms in order to improve treatment of women with asthma.

Introduction

Studies of the epidemiology and natural history of childhood asthma into adulthood demonstrate a change in sex prevalence with age. Asthma usually begins in infancy or early childhood and male gender is one of the principal neonatal risk factors [1]. As illustrated in Figure 1, boys are nearly twice as likely to develop asthma as girls and both a higher male incidence and male prevalence of asthma continue until 16 years of age [2]. In adulthood, however, asthma occurs more frequently among women and female gender is an important risk factor for developing asthma in young adulthood [2]. Moreover, studies consistently find that female gender, atopy, and airway hyperresponsiveness are associated with the persistence of asthma from childhood to adulthood [3].

Intriguingly, severe asthma is also more predominant in women [4]. Asthma in women is associated with excess mortality risk (as compared with the general population) and women with asthma have been found to visit the emergency department for asthma more frequently than men [5]. Subsequent hospitalization rates for asthma reflect the difference in asthma prevalence and severity as observed between men and women [6].

It is still unclear why women are at an increased risk of developing asthma and why they suffer from more severe disease. The observed difference between men and women can be thought of as the product of biological sex differences and sociocultural and environmental differences. Two other papers excellently reviewed the social, cultural, and environmental factors that may contribute to the existing gender differences in asthma [3,7]. This paper therefore explores biological factors like the genetic background, lung structure, sex hormones, and the key players in the allergic inflammation in the lung (eg, the immune system and resident lung cells). Because sex hormones are known to influence these biological factors, we focus especially on their role. By examining the current literature we aim to shed light on possible mechanisms involved in causing gender differences in asthma.

The Role of Sex Hormones in Asthma Susceptibility and Severity

The most compelling evidence for the role of sex hormones comes from the fact that the prevalence of asthma and other atopic conditions is higher in boys before puberty and higher in girls and women after puberty [8]. Because puberty is characterized by a gradual increase in sex hormone levels, their interactions are a likely cause for the observed switch in prevalence. Moreover, early menarche increases the risk of asthma occurrence in girls and women after puberty even further [9]: women with early-onset menarche have higher estrogen levels and are exposed to greater cumulative estrogen and progesterone levels than women with later-onset menarche [10].

Several other findings point towards a role for progesterone and estrogen in the female preponderance for asthma. Among menstruating women with asthma,

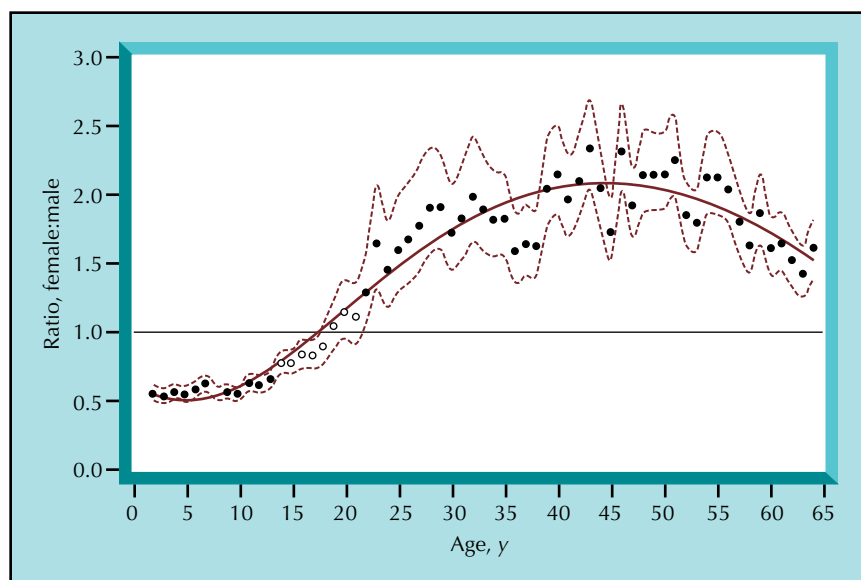


Figure 1. Ratio of female to male asthma patients ages 2 to 64 years. Ratios are presented with 95% confidence intervals (*dashed lines*) and a smoothed curve of the relation between age and female-to-male ratio. The *empty circles* denote sex ratios at ages 14 to 22 years. (From Schatz and Camargo [2]; with permission.)

30% to 40% experience perimenstrual worsening, with increased symptoms and a greater likelihood of hospitalization [11]. Although the mechanism behind perimenstrual asthma remains elusive, it may involve the rise in progesterone and estrogen during the luteal phase. Oral contraceptives (OC) suppress this luteal increase in both sex hormones; despite the lack of randomized controlled trials of hormone therapy, most of the limited number of case reports and studies investigating OC use suggest a small beneficial effect on perimenstrual asthma and mild asthma in general among women [9,11]. However, in women with more severe asthma, OC use may increase asthma severity [12].

The risk of developing asthma with OC use remains unclear. A few studies have addressed this issue and found a decreased risk of developing asthma with increased length of OC use [13•], no effect [14], and an increased risk of current wheeze with OC use [9].

Of interest is the effect pregnancy has on the risk of developing asthma. Pregnancy is generally regarded as a state of helper T (T_H) 2 cell dominance, and is characterized by a gradual increase in progesterone and estrogen levels. Because the hallmark of asthma is a T_H2 -type of inflammation in the lung, pregnancy may predispose women for asthma. Indeed, in a longitudinal study a higher number of pregnancies have been associated with an increased risk of developing adult-onset asthma in women [13•]. In cross-sectional studies, however, this association was not found [15].

With the T_H2 dominance during pregnancy, one would expect an increase in asthma severity in pregnant women with asthma. This does not seem to be the case in women with mild asthma, however: in an equal number of women symptoms worsen, stay the same, or decrease with pregnancy. Only in women with severe asthma, symptoms may increase with pregnancy [16]. Interestingly, the sex of the fetus being carried may influence the effect pregnancy has

on the severity of asthma. Androgens have been shown to have anti-inflammatory effects [17] and spillover from a male fetus may protect the mother. Several studies have found that women pregnant with a female fetus are more likely to have increased symptoms during pregnancy than women pregnant with a male fetus [18•], although this does not appear to lead to more emergency department visits, as was shown in a recent paper [19].

More evidence for a role for estrogen and progesterone comes from the finding that at menopause asthma incidence declines in women when compared to men and younger women [14,20]. This may be linked to a decline in hormone levels, since hormone replacement therapy increases postmenopausal asthma incidence two-fold in women treated for 10 years or more [14,20].

In animal models of asthma, the effects of the different sex hormones seem to be more unambiguous, although the number of studies is limited. As in the human situation, female mice have been found to be more susceptible to allergic airway inflammation than male mice [21,22]. When male mice are castrated, however, they develop the same degree of airway inflammation as female mice, showing a role for androgens in protection against allergic airway inflammation [22]. Progesterone, on the other hand, was found to increase eosinophilic airway inflammation in male mice [23]. The role of estrogen in asthma development is supported by a study in rats showing decreased numbers of eosinophils in the bronchoalveolar lavage fluid (BALF) of oophorectomized female asthmatic rats and a subsequent increase after estrogen supplementation [24].

The general picture that emerges from these clinical and animal studies is that estrogen and progesterone may in some way cause the increased prevalence and severity of asthma in females and that androgens may be protective. However, the influence of sex hormones is most likely a complex interplay among estrogens, progesterone, and

androgens on the different mechanisms involved in the pathogenesis of asthma. This is discussed in more detail in the following sections.

Genetic Factors

One explanation for the increased female susceptibility for asthma is a gender-specific genetic effect. Indeed, some studies have found that several polymorphisms in genes are associated with asthma in women and not in men. For instance, an association has been reported between a cytotoxic T-lymphocyte-associated 4 receptor (CTLA-4) genotype and serum immunoglobulin (Ig) E levels only in women of an adult Chinese population [25]. CTLA-4 may be important in the development of atopy and asthma, as it is involved in a co-stimulatory pathway regulating T-cell activation and subsequent IgE production. The CTLA-4 polymorphism has been shown to alter T-cell activation in human cells. However, so far, no biological mechanism has been described to explain the gender differences observed in this genetic association.

Another example is an association of single nucleotide polymorphisms (SNPs) in the gene for cyclooxygenase-2 (COX-2) in particular in women [26]. COX-2 is an enzyme involved in the production of prostaglandins, mediators of bronchial inflammation. COX-2 (-765 CC) homozygosity is associated with female gender and resulted in an increased capacity of monocytes to produce prostaglandins.

Furthermore, only in women was an association found between SNPs in the gene for defensin β -1 and asthma [27]. Defensin β -1 is expressed by lung epithelial cells and may modulate airway inflammation by a direct antibiotic effect and/or chemoattractant properties.

One study has reported an interesting observation on SNPs in the genes for Toll-like receptor 4 (TLR4) and interleukin-4 (IL-4) [28]. TLR4 is the principal receptor for bacterial endotoxin and plays an important role in the innate immune response to gram-negative bacteria. According to the hygiene hypothesis, early exposure to high endotoxin levels protects against the development of asthma. IL-4 is an important T_H2 cytokine, involved in the induction of airway hyperresponsiveness and IgE synthesis. The study showed that the TLR4 and IL-4 genes have an epistatic effect on the risk for asthma in women, that is, the low endotoxin-responsive allele of TLR4 and the high IgE production allele of IL-4 were found to be a predisposing combination in women. However, this combination of alleles was found in only 13% of female asthmatics.

Finally, Dijkstra et al. [29] found an association between estrogen receptor alpha (ESR1) gene variants and development of bronchial hyperreactivity (particularly in female subjects). It was hypothesized that altered estrogen actions may be involved in abnormal lung development and/or airway remodeling, and that this may be reflected in gender differences in asthma.

Pulmonary Factors

Lung development

In the development of the lung two important aspects should be discerned: maturation and physical growth. Both are affected by gender and may possibly influence asthma susceptibility and severity in females.

During gestation, before birth, males and females have a different pace of lung maturation at the same level of gestation, with males lagging behind [30]. Part of this difference is explained by a delay in the onset of surfactant synthesis in male fetuses, and is caused by an inhibiting effect of dihydro-testosterone and acceleration by estrogen in the period that surfactant synthesis starts [31]. Surprisingly, males seem to catch up with females, and later in prenatal development hardly any difference is observed. No gender differences in surfactant levels in the adult lung have been described so far. Interestingly, though, in adult female mice lacking the estrogen receptor beta (ER β) an accumulation of surfactant protein-A (SP-A) was found [32]. SP-A is also an important mediator in the innate immunity in the lung and has been shown to inhibit allergic responses in murine models of asthma [33]. It is tempting to speculate that estrogen, through ER β , inhibits SP-A production in adult females, which will increase susceptibility for allergic inflammation. This hypothesis needs further research.

Another factor that has been demonstrated to play an important role in the gender differences in lung maturation, and which may influence asthma severity, is epidermal growth factor (EGF). In a mouse model, androgen-induced delay was demonstrated in EGF receptor-binding activity during fetal lung development. This delay in onset of EGF activity was associated with decreased EGF receptor density and occurs at a stage that is critical for alveolar epithelial cell differentiation [34]. The EGF receptor has been shown also to have a proinflammatory role, particularly in severe asthma characterized by neutrophilic inflammation [35], the type of asthma more frequently found in women [4]. The lack of an androgen-induced downregulation of this proinflammatory receptor may add to the increased severity of asthma in women. It is, however, unknown whether androgens can also downregulate the EGF receptor in adult lung tissue as well.

Physical growth of the lungs from birth into adulthood is different between boys and girls and has been cited as a possible explanation for the observed gender switch in asthma prevalence during puberty. At birth boys have relatively narrower, smaller airways in proportion to total lung volume than girls and this may be a risk factor for asthma. During puberty the growth of the airways in comparison to lung parenchyma and air-spaces differs between boys and girls, although the exact underlying mechanism is not understood [36]. Subsequently from 16 years of age, girls/women have smaller, narrower airways in proportion to lung volume than

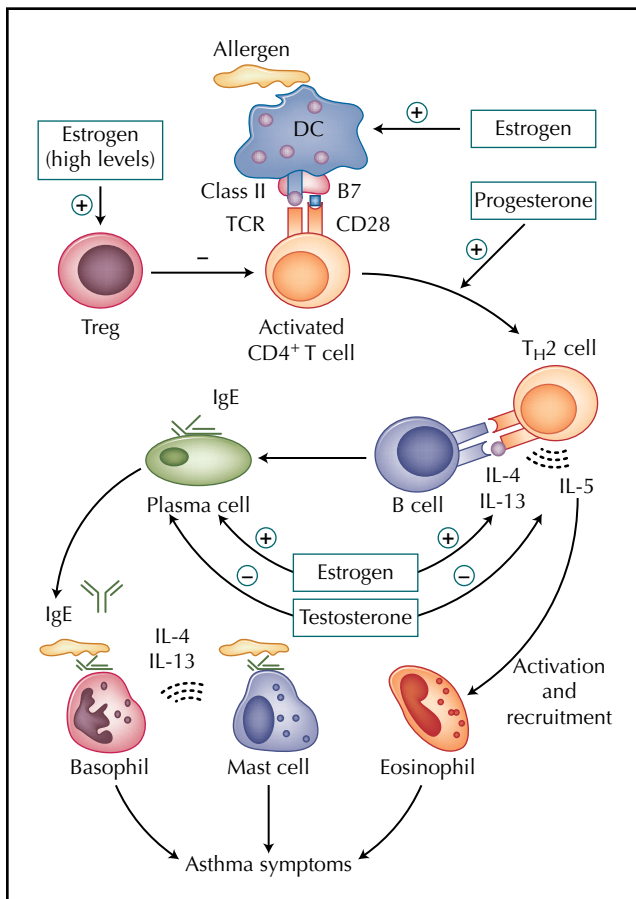


Figure 2. An overview of interactions between allergens and immune cells in asthma and effects of sex hormones on these interactions. Allergens are taken up by dendritic cells (DC) and presented to the T cell receptor (TCR) on CD4⁺ T cells in the context of co-stimulation (B7-CD28 interaction) causing the CD4⁺ T cells to become activated, proliferate, and develop into helper T (T_H) 2 cells. The activated T cells produce T_H2 cytokines (eg, interleukin [IL]-4, IL-5, and IL-13). They also induce B cells to develop into immunoglobulin (Ig) E-producing plasma cells with the help of IL-4 and IL-13. The production of IL-5 leads to the recruitment and activation of eosinophils. IgE binds to the high-affinity IgE receptors on mast cells and basophils, and subsequent interaction with allergens causes the release of inflammatory mediators like histamine, leukotrienes, IL-4, and IL-13. The mediators released from eosinophils, mast cells, and basophils cause the allergic or asthmatic response. Regulatory T cells (Tregs) can inhibit the activated CD4⁺ T cells and help downregulate the immune response.

boys/men of the same age [36]. The theoretical rationale for small airways being a risk factor for asthma, however, has been disputed by physiologists, especially in children because the absolute size of the airways is not much different between boys and girls [37].

Bronchial responsiveness and β 2-adrenoreceptor regulation

The higher incidence of asthma in women during their reproductive years coincides with an increased prevalence in bronchial hyperresponsiveness in women as compared to men [38]. The impact of sex hormones on bronchial

responsiveness has not been studied well, but a few studies have suggested a role for estrogen and progesterone. Estrogen has been found to reduce in vitro carbachol-induced constriction of asthmatic airways by stimulating large-conductance voltage and calcium-dependent potassium channels [39]. This effect appeared to be independent of the genomic actions of estrogen, since this occurred within minutes. Both estrogen and progesterone also potentiated the relaxing effect of the β 2-agonist isoprenaline on porcine bronchial smooth muscle [40]. Only one group has investigated the regulation of β 2-adrenoreceptors by sex hormones in humans using lymphocytes [41]. Although they did not study airway β 2-adrenoreceptors directly, changes in lymphocyte β 2-adrenoreceptor density were shown to closely mirror those in the lung. These studies showed that the increase in sex hormones during the luteal phase of the cycle corresponded with an increase in β 2-adrenoreceptors and a maximal response to isoprenaline. This was most probably due to an effect of progesterone. In women with asthma, however, the upregulation of β 2-adrenoreceptor density and function by progesterone is lost and turned into a downregulation of receptor density and function. This observation may account for a lack of β 2-agonist effects during certain phases in the menstrual cycle and subsequent increased symptoms.

Immunological Factors

Central to the pathogenesis of allergic asthma is an immune-mediated disease in the lungs. Key players in the allergic inflammation are dendritic cells, CD4⁺ lymphocytes, regulatory T cells, and B lymphocytes. Their interactions result in increased levels of T_H2 cytokines IL-4, IL-5, and IL-13, subsequently leading to increases in IgE, influx of eosinophils into lung tissue, and development of bronchial hyperresponsiveness (Fig. 2).

Sex hormones are considered to have important immunomodulatory effects due to observed gender differences in the incidence of many immune-mediated diseases and altered immunological responses during the different reproductive phases in women. Studies in humans and animals suggest that the actions of sex hormones are complex and are directed on both hematopoietic and non-hematopoietic cells. In the following section, we focus mainly on the effects these hormones have on functions of lymphocytes and antigen-presenting cells that are important in allergic inflammation.

CD4⁺ lymphocytes

The expression of estrogen receptors alpha or beta (ER α or ER β) at the protein level has not been rigorously demonstrated on CD4⁺ T cells, which produce cytokines that orchestrate the typical immunological response in asthma [42]. Although some recent studies report expression of ER α at the RNA level using polymerase chain reaction (PCR) techniques [43], it is unclear whether ER is

expressed at sufficiently high levels in CD4⁺ T helper cells. Use of ER-deficient mice has suggested effects on both T_H1 interferon (IFN) γ and T_H2 (IL-4, IL-5, and IL-13) cytokines; these studies are briefly reviewed below.

Effects on IFN γ production

In many models of autoimmune diseases and in vitro studies, estrogen has been found to promote a T_H1 phenotype and increase IFN γ production in CD4⁺ T cells [44]. Based on that evidence one would expect estrogen to inhibit the T_H2-type inflammation in asthma. However, local production of IFN γ appears to have a role in promoting eosinophilia and expression of IL-5 and IL-13 in a mouse model of allergic airway inflammation [45•]. In addition, estrogen was shown to upregulate proinflammatory enzymes inducible nitric oxide synthase (iNOS) and COX-2 in activated murine splenocytes in an IFN- γ -dependent manner [46].

Interestingly, estrogen was also shown to promote IFN- γ production by natural killer T cells (NKT) in both in vitro and in vivo studies [47]. NKT cells have recently been shown to be required for the development of allergic asthma [48,49]. High numbers of NKT cells are more commonly observed in women and this, coupled to their estrogen-induced higher production of IFN γ , may be another mechanism involved in causing gender differences in asthma [47].

In contrast to the enhancing effect of estrogen on IFN γ production from T cells, androgens have been shown to inhibit IFN γ secretion from T cells [50]. Thus, a combination of enhancing effects of estrogen and inhibitory effects of male hormones on CD4⁺ T cells may account for enhanced IFN γ production in females, which may increase certain aspects of the allergic inflammation in the lung.

Effects on IL-4 production

Variations in circulating hormone levels in women have been shown to affect IL-4 production by CD4⁺ T cells in vivo [51]. The most persuasive effect of female sex hormones on tipping the T_H1/T_H2 balance towards T_H2 is during pregnancy at the maternal-fetal interface. Progesterone, which is essential for the maintenance of pregnancy, appears to influence IL-4 production by T cells. In an interesting study, progesterone was shown to upregulate the production of IL-4 from decidual T cells. This subsequently augmented production of leukemia inhibitory factor (LIF) from the T cells, which is essential for embryo implantation [52]. Moreover, women with unexplained recurrent abortions displayed lower IL-4, IL-10, and LIF production by decidual T cells compared to T cell cytokine profiles of women with normal gestation. Furthermore, these same investigators noted the ability of high concentrations of progesterone to increase IL-4 production by established T_H1 cells [52]. The strong influence of progesterone on T_H2 cytokine production was also evident in a different study, in which pregnancy ameliorated

the severity of T_H1-mediated Lyme arthritis due to progesterone-induced IL-4 production [53].

In males, testosterone was found to diminish T_H2 response in splenic T cells, and this was abolished in castrated males [22].

Data on direct effects of estrogen on T_H2 cytokine production by CD4⁺ T cells show only modest effects at best [43]. A review of the literature raises doubts that physiologically relevant levels of estrogen influence T_H2 cytokine production via direct actions on CD4⁺ T_H cells. Collectively, it appears that T_H2 cytokine production is augmented by high concentrations of progesterone and is negatively affected by testosterone. The mechanisms underlying these effects have not been adequately investigated.

Regulatory T cells

With the current interest in regulatory T cells (Tregs) as important regulators of immune responses, studies have explored the possible effects of sex hormones on Treg function. Again, none of the studies were related to the topic of asthma, although Tregs have been shown to play an important role in moderating allergic asthma [54] and may be involved in the increased susceptibility of female mice for asthma [21].

In mice, in vivo administration of estrogen in pregnancy-like concentrations was found to expand the pool of Tregs and enhance their suppressive function, probably to develop fetal tolerance [55•]. However, another study did not detect any change in suppressive functions of Tregs upon estrogen treatment, although they did find increased proliferation of Tregs [56]. These studies suggest that estrogen in pregnancy-like concentrations may be able to reduce allergic airway inflammation by increasing the number of Tregs. So far, no studies have been published studying the effects of non-pregnancy-like concentrations of estrogen or the effects of progesterone and testosterone on Treg function.

B lymphocytes

A large body of literature shows a strong influence of sex hormones on various aspects of B-cell biology including cell development, cell survival, and antibody production. Several studies have documented higher serum antibody titers in women compared to those in men, and one study has even noted changes of IgE titers in the different phases of the menstrual cycle [57]. The higher levels of antibodies in women suggest stimulating effects of female sex hormones and/or inhibitory effects of male hormones on antibody production. In fact, in vitro studies generally support this notion [58]. More recent studies highlight additional levels of regulation by estrogen on B cells that have direct relevance to the higher incidence of both autoimmune and allergic diseases in women. Estrogen has been shown to promote the survival and autoactivation of B cells by influencing the expression of several genes involved in B cell activation and apoptosis [59].

Dendritic cells

Dendritic cells are crucial to the initiation of the adaptive immune response through induction of antigen-specific T-cell responses and by directing the polarization of these responses. Emerging literature suggests an important influence of sex hormones on dendritic cell function. However, limited information currently exists on the effect of sex hormones on dendritic cell function in asthma. In vitro studies in other fields have shown that estrogen promotes the development of functional dendritic cells from murine bone marrow precursors [60] and that the selective estrogen receptor modulators tamoxifen and raloxifene impair dendritic cell differentiation and maturation [61]. Estrogen was also shown to enhance the T-cell stimulating properties of human monocyte-derived dendritic cells [62].

Other Factors: Obesity and Leptin

Another factor emerging as a major risk factor for the development of asthma in women is obesity. Many studies have now shown that asthma incidence in adult women is related to pre-existing obesity and may affect the severity of the disease to a greater degree in women than in men [63]. One of the explanations for this phenomenon is an increase in estrogen levels in obese women due to the aromatase enzyme in adipose tissue converting androgens to estrogen. Another or additional possibility is an effect of leptin, a proinflammatory protein produced by adipocytes. This protein is increased in obesity, but plasma leptin levels have also been found to be higher in women, independent of obesity [64]. A recent study has shown that higher serum leptin concentrations are associated with current asthma in women [65]. A study in mice showed that leptin increased airway hyperresponsiveness and IgE levels in a model of allergic airway inflammation [66•]. This effect of leptin appears to be independent of sex hormones because leptin levels continue to be increased in 80-year-old women as compared to 80-year-old men, even though their estrogen levels were lower than those of the men [67].

Conclusions

Because the disease is complex, no straightforward single mechanism can explain the gender differences found in asthma. Some may be explained by differences on a genetic level, but a large part of this phenomenon constitutes complex interactions between developmental differences, immune function, and resident lung cells caused by sex hormones. Generally speaking, male and female sex hormones appear to exert opposite effects on resident lung cells and immune function that, taken as a whole, render women more susceptible to asthma. Depending on the microenvironment, sex hormones may modulate immune cells, resident lung cells, and other factors that collectively determine the net nega-

tive outcome for women. Further research is essential to understand the mechanisms involved and improve treatment options for women.

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