Estrogen and progesterone decrease *let-7f* microRNA expression and increase IL-23/IL-23 receptor signaling and IL-17A production in patients with severe asthma

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Background: Women have an increased prevalence of severe asthma compared with men. IL-17A is associated with severe asthma and requires IL-23 receptor (IL-23R) signaling, which is negatively regulated by *let-7f* microRNA.
Objective: We sought to Determine the mechanism by which 17β-estradiol (E2) and progesterone (P4) increase IL-17A production.
Methods: IL-17A production was determined by using flow cytometry in T_H17 cells from women (n = 14) and men (n = 15) with severe asthma. Cytokine levels were measured by using ELISA, and IL-23R and *let-7f* expression was measured by using quantitative PCR in T_H17-differentiated cells from healthy women (n = 13) and men (n = 14). In sham-operated or ovariectomized female mice, 17β-E2, P4, 17β-E2+P4, or vehicle pellets were administered for 3 weeks before *ex vivo* T_H17 cell differentiation. Airway neutrophil infiltration and CXCL1 (KC) expression were also determined in ovalbumin (OVA)–challenged wild-type female recipient mice with an adoptive transfer of OVA-specific T_H17 cells from female and male mice.
Results: In patients with severe asthma and healthy control subjects, IL-17A production was increased in T_H17 cells from women compared with men. IL-23R expression was increased and *let-7f* expression was decreased in T_H17-differentiated cells from women compared with men. In ovariectomized mice IL-17A and IL-23R expression was increased and Let-7f expression was decreased in T_H17 cells from mice administered 17β-E2+P4 compared with those administered vehicle. Furthermore, transfer of female OVA-specific T_H17 cells increased acute neutrophil infiltration in the lungs of OVA-challenged recipient mice compared with transfer of male OVA-specific T_H17 cells.
Conclusions: 17β-E2+P4 increased IL-17A production from T_H17 cells, providing a potential mechanism for the increased prevalence of severe asthma in women compared with men. (J Allergy Clin Immunol 2015;136:1025-34.)

Key words: *Estrogen, IL-17A, IL-23 signaling, Let-7f, progesterone, severe asthma*

Sexual dimorphism exists in the setting of asthma, including severe asthma.[1] In children, severe asthma is more prevalent in boys than in girls, but after puberty, there is a change in prevalence, with women being 2 times more likely than men to have severe asthma.[1,2] This change in asthma prevalence suggests a role for sex hormones in asthma pathogenesis, and it is highly likely that sex differences in airway inflammation influence the sex predilection in asthmatic patients. However, the mechanisms that regulate age-related sex differences in asthma prevalence and severity remain unclear.

Asthma has long been associated with $CD4^+$ T_H2-induced inflammation, with increased IL-4, IL-5, and IL-13 secretion and increased airway eosinophil counts.[3-6] Patients with severe asthma might have T_H2 cytokine–mediated inflammation but might also have IL-17–mediated inflammation with increased neutrophil infiltration.[3,7,8] IL-17A levels and T_H17 cell counts are increased in the bronchoalveolar lavage (BAL) fluid of patients with severe asthma compared with values in healthy control subjects or patients with mild asthma.[9,10] In the lungs IL-17A is secreted by $CD4^+$ T_H17 cells, as well as γδ T cells and group 3 innate lymphoid cells (ILC3s).[11-13] Because sex has a role in the regulation of severe asthma prevalence and IL-17A is associated with severe asthma, the focus of our study was to determine the role of sex hormones in $CD4^+$ T_H17 cell differentiation and IL-17A protein expression from $CD4^+$ T_H17 cells by using PBMCs in human subjects and splenocytes in mice. We also wanted to determine the role of sex in IL-17A–mediated airway inflammation and neutrophil recruitment.

T_H17 cells, a distinct subset of $CD4^+$ T cells, secrete IL-17A and IL-17F, as well as other cytokines. IL-23 signaling through the IL-23

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Abbreviations used
AhR: Aryl hydrocarbon receptor
BAL: Bronchoalveolar lavage
E2: Estradiol
FITC: Fluorescein isothiocyanate
GAPDH: Glyceraldehyde-3-phosphate dehydrogenase
ILC3: Group 3 innate lymphoid cell
IL-23R: IL-23 receptor
IRF-4: Interferon regulatory factor 4
miRNA: MicroRNA
OVA: Ovalbumin
P4: Progesterone
PE: Phycoerythrin
qPCR: Quantitative PCR
ROR: Retionic acid–related orphan receptor
SLE: Systemic lupus erythematosus
STAT: Signal transducer and activator of transcription
WT: Wild-type

receptor (IL-23R) is important in maintaining and stabilizing the T_H17 cell phenotype and increasing IL-17A protein expression.[14,15] Recently, *let-7f*, a member of the *let-7* microRNA (miRNA) family, negatively regulated IL-17A protein expression by decreasing IL-23R surface expression on T_H17 cells.[16] $CD4^+$ T cells, including T_H17 cells, express the estrogen nuclear receptors estrogen receptor α and β, progesterone receptors 1 to 4, and androgen receptors through which 17β-E2 (estradiol), progesterone (P4), and testosterone signal, respectively.[17,18] Estrogen signaling in the MCF-7 breast cancer cell line decreased *let-7f* expression, suggesting a role for estrogen in regulating *let-7f* expression.[19] However, the role of sex hormones in regulating *let-7f* expression in $CD4^+$ T cells has not been determined. Based on the increased prevalence of severe asthma in women compared with men and the increased airway neutrophil infiltration associated with IL-17A in patients with severe asthma, we hypothesized that T_H17 cells from women with severe asthma have increased IL-17A production compared with that seen in cells from men with severe asthma.

METHODS

A full description of the methods used in this study can be found in the Methods section in this article's Online Repository at www.jacionline.org. Data are presented as means ± SEMs, with a *P* value of less than .05 being significant.

Recruitment of participants with severe asthma

Patients with severe asthma (aged 18-45 years), as defined by the Severe Asthma Research Program,[20] were recruited from the Vanderbilt University. These patients with severe asthma were clinically stable and not undergoing an exacerbation and continued taking their asthma medications.

Isolation of memory or naive $CD4^+$ T cells from participants

Memory ($CD45RO^+$) or naive ($CD45RA^+$) $CD4^+$ T cells were isolated from PBMCs of patients with severe asthma or healthy subjects (catalog #130-094-125 and #130-094-131; Miltenyi Biotec, Bergisch Gladbach, Germany). Memory $CD4^+$ T cells were then prepared for flow cytometry. Naive $CD4^+$ T cells were activated and differentiated as described below and in the Methods section in this article's Online Repository.

Mice

Wild-type (WT) mice were purchased from Charles River Laboratories (Wilmington, Mass). Sham or ovariectomy surgeries were conducted at 3 to 4 weeks of age. D011.10 transgenic mice were purchased from Jackson Laboratories and bred at Vanderbilt University. Mouse experiments were performed in an Association for Assessment and Accreditation of Laboratory Animal Care–accredited facility with international animal care and use committee approval.

T_H17 cell differentiation from $CD4^+$ naive T cells in human subjects and mice

Naive ($CD45RA^+$) $CD4^+$ T cells were isolated from the PBMCs of healthy subjects (aged 18-45 years) or the spleens of mice by using naive T-cell isolation kits (Miltenyi Biotec catalog nos. 130-094-131 and 130-093-227), activated with anti-CD3 and anti-CD28 antibodies, and differentiated into become T_H17 cells, as previously described and in the Methods section in this article's Online Repository.[21,22]

RESULTS

Patients with severe asthma have an increased number of T_H17 cells in the peripheral blood compared with healthy control subjects.[9,10,23] Therefore we hypothesized that IL-17A production is increased by T_H17 memory cells from women with severe asthma compared with T_H17 memory cells from men with severe asthma. To test our hypothesis, we recruited healthy subjects and patients with severe asthma who were clinically stable. All participants were 18 to 45 years old and did not have a viral or bacterial infection or a T_H17-associated disease (eg, multiple sclerosis, psoriasis, or systemic lupus erythematosus [SLE]). Women were excluded if they were pregnant, breast-feeding, or taking hormonal birth control medications. Patients with severe asthma were defined based on the guidelines used by the Severe Asthma Research Program.[20] There was no significant difference in age, race, body mass index, lung function, total IgE plasma levels, or severe asthma qualifying criteria between women and men with severe asthma (see Tables E1 and E2 in this article's Online Repository at www.jacionline.org). $CD4^+CD45RO^+$ memory T cells were isolated from PBMCs by using negative selection, and we first determined that $IL\text{-}17A^+CD4^+$ memory T_H17 cell counts, as identified based on CCR6 surface expression,[24,25] were increased in patients with severe asthma (n = 29, both women and men) compared with those in healthy adult control subjects (n = 9; Fig 1, *A-C*). Next, we stratified patients with severe asthma based on sex, and women with severe asthma had increased percentages and total numbers of $IL\text{-}17A^+$ memory T_H17 cells compared with men with severe asthma (Fig 1, *D* and *E*).

Patients with severe asthma can also have increased T_H2 cytokine production and increased IL-17A/IL-4–producing $CD4^+$ T cells.[8,26] Therefore total numbers of IL-17A/IL-4 dual-producing, $CCR6^+$ memory T_H17 cells were also determined, and no difference was found (see Fig E1, *A-C*, in this article's Online Repository at www.jacionline.org). There was also no difference in IL-4 production in $CD3^+CD4^+$ memory T cells or total IgE plasma levels between women and men with severe asthma (see Fig E1, *D* and *E*, and Table E2). Furthermore, we found no association between total IgE plasma levels and $IL\text{-}17A^+$ memory T_H17 cell numbers (see Fig E1, *F*). Collectively, these data showed that women with severe asthma had increased numbers of IL-17A–producing T_H17 cells compared with men with severe asthma, but the mechanism or mechanisms remained unclear.