

Title: Sex differences in the lung immune response to environmental agents

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Abstract: Sex differences in respiratory physiology have been identified. Likewise, accumulating evidence suggests that gender affects the incidence, susceptibility, and severity of lung disease. For example, asthma prevalence is higher in boys than girls. This pattern reverses in puberty when the prevalence becomes higher in women than men, and then reverts again to a male-predominant pattern after menopause. In addition, menstrual cycle variations in asthma severity are well established, postmenopausal women receiving hormone replacement therapy have significantly less airway obstruction than those not receiving it, and females with Turner's Syndrome (low circulating estrogen) exhibit increased airway responsiveness that is reduced with estrogen therapy. We hypothesized that circulating levels of sex hormones affect the inflammatory response to environmental agents. We tested our hypothesis in mouse models of acute inflammation (exposure to 2ppm of ozone for 3h) and chronic inflammation (exposure to house dust mite allergens for 5 weeks) by comparing the responses of intact vs. gonadectomized male/female mice. We verified the presence of peri-bronchial inflammation in lung sections, we identified differences in inflammatory gene expression, and in airway reactivity by measuring the sensitivity of animals to contractile agonist challenge using a rodent ventilator. We found significant differences in airway resistance and hyperresponsiveness in males vs. females with both challenges. In both sexes, gonadectomy and hormone replacement affected lung function and expression levels of inflammatory genes. Together, our results indicate that circulating levels of sex hormones affect lung function and prime the lungs for differential inflammatory responses following exposure to environmental agents. This information may help identify mechanisms associated with gender disparities in asthma incidence and severity, and variations of symptoms observed in men and women throughout life.

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Title: Sex steroid signaling in the airway

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Abstract: Asthma is more common in pre-pubescent males, but increases in women and aging males, highlighting roles for sex steroid effects in airways. A limitation to understanding how sex steroids influence asthmatic airways is their complex, cell- and context-dependent effects. Effects on bronchial epithelium and airway smooth muscle (**ASM**) are relevant, given their roles in modulating airway tone and structure. The focus should probably be on estrogens given increase in asthma among young women and that progesterone does not modulate estrogen effects. Whether and how estrogens are protective or deleterious in asthmatic airway is not clear. Emerging data show that **1)** Human ASM expresses ER α and ER β ; **2)** Estrogens non-genomically reduce ASM intracellular calcium ([Ca²⁺]_i) responses to agonist and increase cAMP, overall aiding bronchodilation. Our data in human ASM show that: **1)** Asthmatic or cytokine-exposed ASM express more ER β than ER α , suggesting a shift in ER profile; **2)** With inflammation, ER β functionality is enhanced, and has a suppressive effect on [Ca²⁺]_i, ASM proliferation and fibrosis; **3)** ER α and ER β signaling diverge in inflamed or asthmatic ASM, with differential effects on cAMP vs. NF κ B and p38 MAPK. In mice, estrogens downregulate airway hyperresponsiveness. In models of allergic asthma, ASM ER β is increased (less so in epithelium), while conversely absence of ER β results in greater airway thickening, reactivity, and ASM expression of Ca²⁺ regulatory and fibrosis proteins. Conversely, ER β -specific agonists blunt airway reactivity and remodeling, and ASM expression of fibrosis proteins. Overall, emerging data highlight the need for further research into mechanisms by which estrogens affect the airway, the cell types involved, and specific roles of different receptors. Here, ER β may take an “anti-inflammatory” role, setting the stage for exploration of ER β in helping explain conflicting data on estrogens in asthma.

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Title: Circulating Sex Hormones as Critical Regulators of the Lung Immune Response

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Abstract: Exposure to ground-level ozone, an oxidative pollutant, causes lung inflammation, which can lead to pulmonary injury and impair lung innate immunity. There is emerging evidence that pulmonary diseases such as asthma affect women disproportionately with a greater degree of severity than men. In addition, most hospitalizations for asthma in women occur during the luteal phase or around the peri-menstrual stage. However, the combined effect of ozone and sex hormones in respiratory mechanics is unclear. Here, we hypothesized that circulating estrogen levels can regulate pulmonary function and lung mechanics following ozone exposure. We performed gonadectomy and hormone replacement (17 β -estradiol, 2 weeks) in a group of adult male and female C57BL/6 mice. In control females, the stages of the estrous cycle were monitored by daily vaginal smear, and confirmed by serum sex hormone levels. We exposed animals to 1 ppm of ozone or filtered air (FA) for 3 hours, and we compared lung function 24h after exposure with a methacholine challenge (MCh; 0 - 50 mg/ml) using the FlexiVent system. We observed significant changes in respiratory parameters (Ers, H, Rrs, R_N, G) in males and females, and in females exposed to ozone at different stages of the estrous cycle, at the two highest MCh concentrations. The pressure-volume curves obtained demonstrated similar MCh concentration-dependent changes. Gonadectomized males exposed to ozone had higher Rrs and Ers than females and males exposed to FA, and treatment with estradiol ameliorated these effects. Surprisingly, female mice in the metestrus and diestrus stages exposed to FA had higher Rrs and Ers values than when compared to the proestrus and estrus stages. Contrarily, exposure to ozone caused a decrease in parameters (Ers, Rrs) during metestrus and diestrus stages, but a slight increase of these in the proestrus and estrus stages. Our results indicate that pulmonary function following ozone exposure can be affected by circulating hormone levels. Future studies examining diseases associated with environmental pollutants should consider the women's menstrual cycle.

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