Immunity Comes to Play in the "Sex Paradox" of Pulmonary Arterial Hypertension

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Once made equal to a man, woman becomes his superior.

—Socrates

Attempting to give a biological twist to Socrates quote, that is, how women, once equal, evolve to be superior to men, epigenetics comes to mind. Through evolution, women have evolved to be fitter than men when it comes to resistance to infections, the biggest human killer in history.[1] They do have a more responsive immune system and new (though provocative and preliminary) evidence now suggests that this may be because of incomplete inactivation of 1 of their 2 X chromosomes, which is normally epigenetically silenced to offer a gender-balanced gene expression.[2] The X chromosome hosts many genes essential to the regulation of the immune system.[2] Females can carry 2 alleles of some X chromosome genes (and thus have higher levels of the expressed proteins), compared with men that can only have one allele from their single X chromosome, contributing to a more fit immune system.

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But there is a price to pay. Their stronger immune system, when goes wrong, makes them more prone to inflammatory or autoimmune diseases, a well-recognized clinical fact.[3] Lymphocytes from women with autoimmune diseases can simultaneously express both alleles of X chromosome genes encoding proteins like CD40L, CXCR3, or FoxP3, via changes in their X chromosome inactivation machinery.[2] These proteins are important for regulatory T cell (Treg) development, activation, and trafficking and they have been indirectly implicated, in early studies, in the endothelial cell dysfunction of pulmonary arterial hypertension (PAH),[4,5] one of the earliest events in the natural history of the disease. The role of immunity in the pathogenesis of PAH is being increasingly recognized and perivascular infiltration of remodeled pulmonary vessels with immune cells is one of the strongest histological features of PAH.[6] Could these observations be relevant to another mystery in the field of PAH?

Although all forms of PAH are more common in females than males, females develop a somewhat milder form of PAH associated with better outcomes.[7] This so-called sex paradox has been heavily studied and attempts to explain it have mainly focused on the role of sex hormones. Estrogens have been implicated in the higher prevalence of many diseases of dysregulated immunity, including the autoimmune diseases systemic lupus erythematosus, scleroderma, or multiple sclerosis. Several studies have shown that some estrogen metabolites (like 2MeO-estradiol) promote vasorelaxant, antiproliferative, and proapoptotic signals in pulmonary vascular cells, while others (like 16-α-OH-estradiol) do the opposite.[8] Because key enzymes that determine the relative levels of the former versus the latter (CYP1B1 and catechol-O-methyl-transferase) are expressed in the lungs, this imbalance has been proposed to be the basis of the differential effects of estrogens on male versus female pulmonary vascular cells.[8] There is much more complexity in this story, however, considering that these differences are tissue and context-dependent and associated with dynamic changes in estrogen receptors both in the plasma membrane and the nucleus. This interplay of estrogen metabolites and receptors in human and animal PAH has been well-reviewed by Lahm et al.[7] Several conflicts among these studies suggest that other factors must contribute to this paradox. Reliance on the effects of sex hormones has failed to fully explain the sex paradox of PAH.[7,8]

Nonhormonal factors, like those resulting from incomplete X chromosome inactivation, may be relevant to this. This notion is supported by observations in experimental studies from other inflammatory diseases, where mice with an XY complement of chromosomes (regardless of gender) develop more severe disease than an XX complement ones independently of sex hormones.[9] Alternatively, the Y chromosome may also exert direct protective functions to prevent the development of pulmonary hypertension independently of sex hormones, as recently suggested.[10]

These estrogen-independent mechanisms seem to be directly relevant to Treg cell function because females express higher levels of proteins critical to Treg function, as discussed above. Although Treg cells have previously been implicated in PAH biology in general,[11,12] a new study in this issue of *Circulation Research*, attempts to directly link for the first time Treg cell function in the PAH sex paradox.[13]

The authors show that intact Treg function in females is more critical for the maintenance of immune homeostasis than in males, suggesting that Treg in females play a protective role in suppressing immune dysregulation, inflammation, and vascular remodeling in PAH models. Female athymic nude rats (that lack Treg cells) treated with the VEGF inhibitor SU5416 developed worse pulmonary hypertension, with more severe right ventricular hypertrophy and fibrosis, than athymic male nude rats. The loss of Treg function in females was associated with a substantial reduction in the levels of several factors that

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either protect against endothelial cell apoptosis or promote cell survival and vasorelaxation, all important features of PAH. These factors included HO-1 (heme oxygenase 1) and the immune-regulatory protein PDL-1 (program death ligand-1), both promoting cell survival; and key enzymes critical for the biosynthesis of PGI_2 (prostaglandin I_2), a critical pulmonary vasorelaxant, that also has anti-inflammatory properties. Immune reconstitution with Treg restored the expression of HO-1, PDL-1, PGI_2 synthetic enzymes, and PGI_2 serum levels. Coculture of pulmonary endothelial cells with Treg promoted an estrogen receptor-dependent release of PGI_2 and the anti-inflammatory cytokine IL (interleukin)-10. Direct coculture of Treg with right ventricular microvascular endothelial cells also resulted in increased expression of HO-1, PDL-1, and estrogen receptors. This is one of the few studies that have examined the effects of an immune mechanism in both the pulmonary vasculature and the critically important right ventricle.

The study suggests that female Treg interact with endothelial cells to protect them from apoptosis (which is considered one of the earliest events in PAH pathogenesis) and enhance their production of vasorelaxant and prosurvival factors. It still remains to be determined whether this Treg-endothelial cell interaction is direct (requiring contact versus a secretable factor) and whether it is dependent or modified by hormonal factors. Future studies targeting endogenous (eg, oophorectomy) or exogenous estrogen metabolites will be important in defining how Treg protect from pulmonary hypertension in the presence of estrogen. Certainly, at least one direct clinical implication may be that the benefits from prostacyclin analogs, perhaps the most effective PAH therapy, may differ in female versus male PAH patients.

This is a welcome new window to the pathogenesis of this mysterious disease in general and directly contributes to the study of the PAH sex paradox. How does it relate to other evolving theories in PAH? The metabolic theory suggests that the proproliferative and antiapoptotic diathesis in PAH is in part driven by vascular cell mitochondria.[14] In addition, inflammation can be directly triggered by mitochondria, through the induction of the NLRP3 inflammasome.[14] Importantly, the function of T cells is strongly related to their mitochondria function. For example, a mitochondrial suppression of oxidative phosphorylation (ie, a decrease in glucose oxidation and switch to glycolysis) is a necessary step that precedes T cell activation.[15]

Are female mitochondria different than male mitochondria? They may be! Mitochondria are passed to the offspring only by the mother. Female mitochondria can respond to evolutionary pressure because they are passed on to the offspring. But male mitochondria are not responsive to such pressures because they are locked in the host cell and cannot be passed on. Mutations in the mtDNA (which only encodes 13 proteins but critical to the regulation of membrane potential and ATP production) occur fast mainly because of its proximity to the main source of reactive oxygen species in the cell (ie, the mitochondrial electron transport chain). There are mtDNA mutations that may be beneficial to female but detrimental to males. These would respond to positive selection bias only in females and thus accumulate in males.[16] In such cases, the male nuclear genome will respond trying to defend to the unfriendly mitochondria with genetic or epigenetic remodeling,

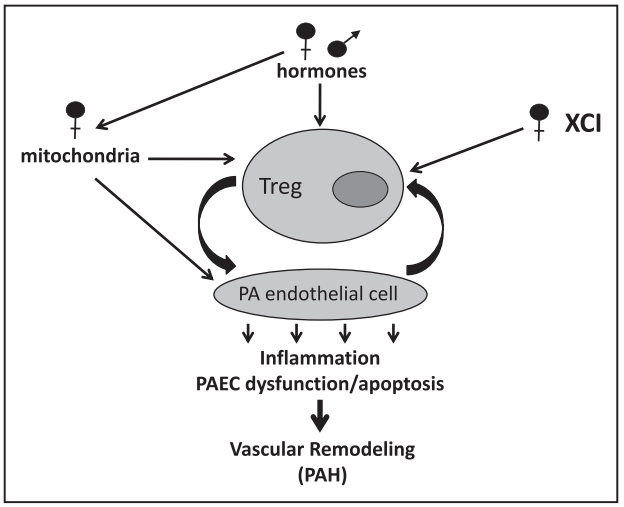

Figure. New players in the sex paradox in pulmonary arterial hypertension (PAH). Most studies so far have implicated sex hormones in the explanation of the sex paradox in PAH. Additional potential players may include vascular mitochondria and the X chromosome inactivation (XCI) machinery. New evidence in this issue of the journal points to a central role of regulatory T cells (Treg) in potentially integrating the above signals and limiting the progression of PAH (see text).

disrupting the normal mitochondria-nucleus communication.[16] In other words, there is a form of a battle of the sexes taking place at the mitochondrial level, in which the males are clearly in the defensive. This provocative hypothesis is not yet fully studied in mammalian cells. It is intriguing however that oocytes have mitochondria with slower metabolic activity, lower ROS production and different ultrastructure than sperm cell mitochondria.[17]

Genetic mechanisms driven by X chromosome epigenetics and mitochondrial evolution may help explain the sex paradox in PAH, with hormones playing a modifier role (Figure). For example, both estradiol and estrogen metabolites can regulate mitochondrial function through estrogen receptor-mediated nuclear gene transcription (ie, transcription of mitochondria master regulators like NRF-1 or Tfam, which regulate both mitochondrial function and biogenesis) or indirectly through plasma membrane estrogen receptor-dependent signaling. Intriguingly, both α and β estrogen receptors have been shown to be active within mitochondria in many cell types and tissues.[18]

In summary, the reliance on estrogen metabolites has not solved the sex paradox in PAH. The article by Dr Nicoll's group, opens a new window in PAH, pointing directly to a major target of the X-chromosome remodeling, the Treg cells, with potentially important implications in cardiovascular biology beyond PAH. And perhaps Socrates quote may be closer in getting a biological explanation.

Disclosures

None.

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