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Editorial

Coxsackievirus B3-Induced Myocarditis: New Insights Into a Female Advantage

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See article by Zhou et al., pages 492–501 of this issue.

Myocarditis is an inflammatory cardiomyopathy that is characterized by the inflammation of cardiac muscle cells.[1] Although the disease itself can be life-threatening, its prognosis as well as pathogenesis vary widely. Myocarditis can be acute, subacute, or chronic and can feature either focal or widespread myocardial damage.[2] Unsurprisingly, this leads to a variable degree of myocardial injury that can range from a fully recoverable syndrome to systolic dysfunction and dilated cardiomyopathy that results in death or requires heart transplantation.[2,3] The gold standard for diagnosis requires endomyocardial biopsy and histopathological techniques to identify the characteristic infiltration of mononuclear cells into the myocardium and evidence of necrosis that is not secondary to myocardial ischemia.[4] Still, the diagnosis of myocarditis is challenging because of sampling errors during biopsy and the rarity of performing biopsies globally, so that the actual incidence of disease is unclear.[3]

Myocarditis can arise in many settings including exposure to autoimmune diseases and toxic agents, but it is widely recognized that microbial infections are a major cause of disease.[3] Although diverse agents such as bacteria, protozoa, helminths, and fungi have been implicated, most cases are attributable to viral infections.[2] One such, the coxsackievirus B3 (CVB3), is a relatively common enterovirus that typically leads to mild upper respiratory and gastrointestinal illnesses and that, in small number of unfortunate individuals, can cause myocarditis.[5] Myocardial dysfunction is hypothesized to arise from direct effects of CVB3 on cardiac myocytes, the activation of antimicrobial host-defense mechanisms and/or the production of heart-reactive autoantibodies.[2,5] Even so, the underlying mechanisms responsible for myocardial dysfunction after CVB3 infection are not fully understood. That the incidence and severity of CVB3-induced myocarditis is greater in men than in women implicates sex-steroid hormones in disease pathogenesis.[2,6] Interestingly, this female advantage is also seen in mouse models of CVB3 myocarditis. Young adult female mice are protected from myocardial inflammation, injury, and death whereas male mice are not.[2] These findings suggest that estrogen might play a protective role in the setting of viral myocarditis.

In this issue of the *Canadian Journal of Cardiology*, Zhou and colleagues explore mechanisms involved in the relationship between estrogen and CVB3-induced myocarditis in male and female mice.[7] The authors show that adult male mice exposed to CVB3 exhibit a rapid and severe myocarditis characterized by profound inflammatory cell infiltration, widespread necrosis, and the release of cardiac troponin I within 7 days of infection. CVB3 infection also increased mortality in the male group. In contrast, there was significantly less myocardial damage in adult female mice infected with CVB3 over the same time frame. Interestingly, this sex difference is only apparent after puberty, because no male-female differences are seen in sexually immature mice. When male mice are injected with 10 μg of estradiol 1 day after CVB3 exposure, they exhibit a phenotype similar to that of female mice, and their survival is significantly increased. A clear strength of the work is the use of sham-operated and ovariectomized (OVX) female mice to confirm their hypothesis that estrogen is a key player in CVB3-induced myocarditis. They show that CVB3 infection of OVX female mice causes a marked increase in histological signs of myocarditis, an increase in cardiac troponin I release, and greater mortality compared with sham control mice. Together these data provide convincing evidence for a beneficial role for estrogen in viral myocarditis.

Previous work from this group has implicated natural killer (NK) cells (immune cells that clear viruses by direct cytotoxic effects as well as secretion of proinflammatory cytokines) in sex differences in the severity of CVB3-induced myocarditis in the mouse model.[8] They showed less infiltration of pathological interferon (INF) γ-positive (INF-γ^+) NK cell in hearts from young adult female mice compared with age-matched male mice.[8] This is important. Although the cytokine INF-γ is secreted by NK cells to help protect against viral infections, aberrant INF-γ production can promote inflammation and tissue damage.[9] In their new study,[7] the authors explore molecular mechanisms responsible for the beneficial effects of estrogen on cardiac INF-γ^+ NK cell infiltration in CVB3-

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induced myocarditis. They show that CVB3 increases INF-γ production in isolated NK cells and that this is inhibited when NK cells are exposed to estradiol. In support of this, they show marked infiltration of INF-γ^+ NK cell in hearts from male as well as OVX female mice compared with intact female mice after CVB3 infection. Critically, INF-γ^+ NK cell infiltration is attenuated by estradiol treatment in both models. Their work also shows that estrogen inhibits infiltration of INF-γ^+ cells in the heart by downregulating the expression of T-bet. Why is this significant? Because T-bet (a key transcription factor linked to INF-γ) turns on genes that boost the production of INF-γ.

This new work by Zhou and colleagues is important. Viral infection is a major cause of inflammatory cardiomyopathies and there are few treatment options available.[3] Epidemiological work from the Global Burden of Disease Study estimated 339,500 deaths attributable to myocarditis and cardiomyopathy in 2016 alone.[10] Despite clinical and preclinical evidence that CVB3-induced myocarditis is worse in men than in women,[2] until now there has been limited mechanistic understanding of this female advantage. It is now clear that estrogen is a critical determinant of the severity of CVB3-induced myocarditis and that this is mediated, at least in part, by pathological effects of INF-γ secretion from NK cells. Estrogen receptors α and β are both present on NK cells,[2] so this work suggests that estrogen might regulate NK cell function and it sets the stage for additional studies in this area. Still, some important questions remain. As Zhou et al.[7] make clear, NK cells can induce cardiomyocyte death (apoptosis) by other means, such as the granzyme B/perforin pathway; beneficial effects of estrogen on CVB3-induced myocarditis might be more complex than outlined. There is also some evidence for an increased incidence and younger age of onset of myocarditis in men than women, suggesting the susceptibility might be higher in women after the onset of menopause.[2] In support of this, older female mice exposed to CVB3 appear to exhibit a proinflammatory phenotype similar to that seen in age-matched male mice.[2] Studies from our group and others show that age itself has sex-specific detrimental effects on the structure and function of the heart and this might affect the expression of heart disease differently in men and women.[11] Still, the effect of age, menopause, and hormone replacement therapy on the pathogenesis of viral myocarditis is not well understood and more work in this area could be informative.

The work of Zhou et al.[7] also highlights the importance of conducting studies in both sexes, an idea that is happily gaining traction now that granting agencies throughout the world have begun to mandate the use of male and female models in clinical as well as in preclinical work.[12-15] In that context, it is worth drawing to attention that the critical role of estrogen in CVB3-induced myocarditis would not have been identified if studies had used only male mice, as has been the widespread practice until recently. Likewise, the role played not just by age itself but by the widespread accumulation of age-related deficits can only be expected to be revealed if older animals are also studied.[16]

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