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EDITORIAL COMMENT

Taking Sex Seriously

An Oft-Overlooked Biological Variable*

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The inflammasome, a supramolecular cytoplasmic structure comprising multiple subunits, senses danger in various guises and activates its ultimate component, caspase-1, to generate mature interleukin (IL)-1β and IL-18 from their inactive precursors (**Figure 1**) (1). The inflammasome participates in many inflammatory diseases, including those of the cardiovascular system. Diseases precipitated by crystalline structures such as monosodium urate in gout and cholesterol monohydrate in atherosclerosis, among other pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), trigger the inflammasome. Many lines of evidence converge to demonstrate the pivotal importance of the inflammasome and its products in human diseases. For example, rare genetic disorders associated with constitutive gain of function of the NLRP3 inflammasome cause cryopyrin-associated periodic syndrome or Muckle-Wells syndrome. Neutralizing IL-1β has proven transformative in the treatment of these diseases. CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcomes Study) provided the first proof of the success of targeting inflammatory pathways in cardiovascular disease by administration of a neutralizing antibody for the inflammasome product IL-1β in individuals with established coronary artery disease and persistent inflammation despite standard-of-care therapy, including high-intensity statin treatment (2). Downstream of IL-1, IL-6 has proven causal in human atherothrombosis based on concordant Mendelian randomization studies in humans. These convergent strands of evidence all point to a pivotal role of the inflammasome in human disease and coronary heart disease in particular.

We routinely use mice to test hypotheses regarding disease mechanisms. The ready genetic manipulation in this species and the existence of inbred strains facilitate our investigative quest to unravel disease pathogenesis. Exploration of the role of the inflammasome using such techniques has nonetheless proven confusing. A seminal study by Duewell et al. (3) assessed the effect of genetically induced loss of function of the NLRP3 inflammasome in experimental atherosclerosis in mice; they reported an attenuation of atherogenesis in mice. Menu et al. (4) undertook similar experiments, albeit with a different intensity of a cholesterol-enriched diet, and showed no such moderation of atheroma formation. The Duewell et al. (3) experiments clearly specified the use of female mice. The null findings reported by Menu et al. did not specify the sex of the animals studied. In this issue of *JACC: Basic to Translational Science*, Chen et al. (5), in a carefully designed head-to-head comparison, showed that loss of function of the NLRP3 inflammasome attenuates atherosclerosis in female but not in male mice

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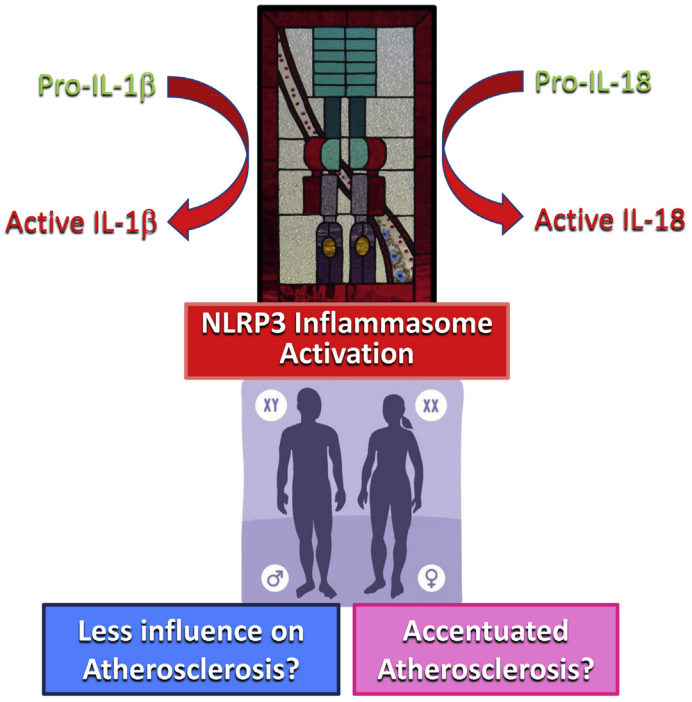
FIGURE 1 The NLRP3 Inflammasome, Depicted Here Artistically in Stained Glass, Processes the Inactive Precursor Forms of the Proinflammatory Cytokines IL-1 Beta and IL-18 to the Mature Active Mediators



Data presented by Chen et al. (5) suggest that in mice, inflammasome activation accentuates atherosclerosis preferentially in females. These findings highlight the need to consider sex as a biological variable in animal experiments and reinforce the necessity of including women in clinical trials. These experimental findings should stimulate analyses to seek sex differences in inflammatory pathways in humans. The stained glass image shows a long-axis view of the NLRP3 inflammasome with an atherosclerotic artery in the background (a design conceived by Dr. Peter Libby and biologist/stained glass artist Dr. Joel Kowit, who created it). The image of the silhouettes of a man and a woman is from the National Institutes of Health *News in Health* and was drawn by illustrator Alan Defibaugh (https://newsinhealth.nih.gov/2016/05/sex-gender). IL = interleukin.

(5). Their careful observations help clarify the seemingly disparate findings of the 2 prior studies discussed.

Furthermore, Chen et al. (5) found that castration of male mice rendered them sensitive to inhibition of atherosclerosis by NLRP3 inflammasome loss of function. Ovariectomy of female mice rendered atherosclerotic lesion formation insensitive to inflammasome loss of function. These latter ablative experiments implicated sex hormones in the dependence of inflammatory processes mediated by the inflammasome. Nonetheless, these intriguing experiments do not establish the molecular mechanism by which gonadal products can modify the responses of experimental atherosclerosis to modulation by the inflammasome and its products.

We have long appreciated that autoimmune diseases can occur more frequently in women than men. Perhaps these mouse experiments lend some insight into possible mechanisms that underlie the greater susceptibility of women to certain inflammatory diseases such as lupus erythematosus, rheumatoid arthritis, and the like. In addition to sex hormones, sex chromosomes can influence these inflammatory diseases. The X chromosome carries many genes involved in immune functions, including CD40 ligand (CD154) and IL-1 receptor-associated kinase-1. Moreover, the X chromosome contains 10% of microRNA in the genome. Many of these noncoding RNAs may modulate cardiovascular diseases and immune responses (6). These various observations highlight the need to consider sex seriously as a biological variable. Too many experimental studies use animals of only 1 sex or do not specify or assess response in both sexes. The story of the inflammasome in atherosclerosis recounted here underscores the need to do so (5). Many studies have used male animals in a stated attempt to avoid potential confounding by cyclic hormone changes in females. Is this justification for avoiding the study of female animals an extension of paternalistic thinking, or even of remnants of scientific misogyny persisting from times of yore? Rather than regarding the fluctuations as confounders, and thus excluding study of female mice or mixing the sexes willy-nilly, we should embrace the study of differential responses between the sexes to derive insight into biologic mechanisms of capital importance to half of our population. We must ask ourselves critically if excluding 1 sex or another in the design of experimental studies has a strong biological justification or merely represents a convenient extension of habit or of traditional practices.

The recent heightened sensitivity of the importance of sex as a biological variable both by funding agencies and scientific journals has begun to remediate this deficiency in our enterprise. Data assembled in a recent important compilation in *Arteriosclerosis, Thrombosis, and Vascular Biology* showed that <40% of publications in 2017 reported both sexes or provided justification of why only 1 sex was studied (7). By the end of 2018, this number was approaching 100%. Most clinical trialists today strive to achieve greater inclusion of women and underrepresented minorities in clinical trials. Although we seem to be improving in both laboratory and clinical studies in this regard, we must strive to do better to address both sexes experimentally. Funders should provide sufficient budget to permit this effort. The sponsors of clinical trials should provide the resources required to enroll representative numbers of

women and members of minority populations. Such efforts can yield both expected and unexpected benefits. The inclusion of some 6,000 women in JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin), a primary prevention trial with rosuvastatin, helped to dispel the myth that women do not benefit from statins (8). The fibrate arm of ACCORD (Action to Control Cardiovascular Risk in Type 2 Diabetes) showed directionally opposite point estimates of event reduction in men and in women (9). The recent PARAGON-HF (Prospective Comparison of ARNI with ARB Global Outcomes in HF With Preserved Ejection Fraction) study showed a possibly greater benefit of a combination of an angiotensin-receptor blocking agent with a neprilysin inhibitor in women than in men with heart failure with preserved ejection fraction (10). This finding has important implications for future clinical trials. Thus, in both the laboratory and in the clinic, we have no excuse not to make every effort to include both males and females in our investigations. We stand to learn more about underlying mechanisms of health and disease and about how to provide optimum care for each individual we treat in the clinic.

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