Preclinical Research

Sex-Specific Effects of the Nlrp3 Inflammasome on Atherogenesis in LDL Receptor-Deficient Mice

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VISUAL ABSTRACT

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HIGHLIGHTS

- In this study we observed sex-specific effects of the NLRP3 inflammasome on atherogenesis in LDLR-deficient mice, with NLRP3 inflammasome playing a more prominent role in atherosclerosis in female mice than in males.
- Sex hormones may be involved in NLRP3 inflammasome-mediated atherogenesis and may underlie differential responses to anti-NLRP3 therapy between males and females.
- Testosterone may play an inhibitory role by blocking NLRP3 inflammasome and inflammation in atherogenesis, whereas female sex hormones may promote NLRP3 inflammasome-mediated atherosclerosis.
- The results of the present study may help design future clinical trials, with the objective to personalize cardiovascular care for men and women.
In the LDLr<sup>-/-</sup> mouse model of atherosclerosis, female Nlrl<sup>-/-</sup> bone marrow chimera and Nlrp3<sup>-/-</sup> mice developed significantly smaller lesions in the aortic sinus and decreased lipid content in aorta en face, but a similar protection was not observed in males. Ovariectomized female mice lost protection from atherosclerosis in the setting of NLRP3 deficiency, whereas atherosclerosis showed a greater dependency on NLRP3 in castrated males. Thus, castration increased the dependency of atherosclerosis on the NLRP3 inflammasome, suggesting that testosterone may block inflammation in atherogenesis. Conversely, ovariectomy reduced the dependency on NLRP3 inflammasome components for atherogenesis, suggesting that estrogen may promote inflammasome-mediated atherosclerosis. (J Am Coll Cardiol Basic Trans Science 2020;5:582-98)

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Role of Sex in IL-1β and Atherosclerosis

suggest a sex-specific response to IL-1β, indicating that a smaller sample size was needed for females to achieve an equal therapeutic benefit as males. These results suggest a sex-specific difference in the therapeutic responses to IL-1β inhibition, where females may be more responsive than males. Although the results of the CANTOS trial are a milestone in cardiovascular medicine, the safety concerns and potentially prohibitive cost make it unlikely that canakinumab will ultimately be used for secondary prevention.

Therefore, finding ways to identify subsets of patients who derive maximum benefits from canakinumab (or other anti-inflammatory agents) is critical. Here, we investigated the role of sex in NLRP3 inflammasome-mediated inflammation in atherosclerosis as a first step toward identifying these patient subsets.

Methods

Animal Studies. All animal experiments were performed according to the guidelines and approved protocols (Protocol #8299) of the Cedars-Sinai Medical Center Institutional Animal Care and Use Committee. Cedars-Sinai Medical Center is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care and abides by all applicable laws governing the use of laboratory animals. Laboratory animals are maintained in accordance with the applicable portions of the Animal Welfare Act and the guidelines prescribed in the U.S. Department of Health and Human Services publication, Guide for the Care and Use of Laboratory Animals.

Mice. All mice were on the C57BL/6 background for these studies. Both male and female Nlrp3−/−/Ldlr−/− and Ldlr−/− mice were used (42). For bone marrow (BM) transplantation, BM from wild-type, and Nlrp3−/− mice was transplanted into irradiated Ldlr−/− mice. After recovery (6 weeks), chimeric mice were placed on a high-fat diet (HFD) containing 0.15% cholesterol (Harlan Teklad) for 12 weeks (42,43).

Castration. One week before HFD, Nlrp3−/−/Ldlr−/− and Ldlr−/− male mice of 8 weeks of age underwent castration (CAS) or sham-surgery. Mice were maintained on inhalation anesthesia (1.5% isoflurane) via nose-cone. Before the start of the surgery, carprofen (5 mg/kg body weight) was administered subcutaneously. The area between the penis and the anus was shaved and cleaned with betadine followed by alcohol to disinfect the scrotum. The area between the penis and the anus was made and the testicles were exteriorized. Testicular arteries were tied off using resorbable Vicryl sutures before removing the testes. Once the testes were removed, the wound was sealed with 2 nylon sutures. Following spontaneous movement, buprenorphine (0.5 mg/kg body weight) and 300 µl of warm saline were administered subcutaneously. In sham-operated mice, both the skin and inner skin membrane between the penis and...
anus were incised. The testes were drawn out and placed back and the wound was sealed with interrupted nylon sutures.

**OVARIECTOMY.** One week before HFD, Nlrp3⁻/⁻ Ldlr⁻/⁻ female mice of 8 weeks of age underwent ovariectomy (OVX) or sham-surgery. Mice were maintained on inhalation anesthesia (1.5% isoflurane) via nose-cone. Before the start of the surgery, carprofen (5 mg/kg body weight) was administered subcutaneously. The area below the ribs was shaved and cleaned with betadine followed by alcohol. This area was then lifted with forceps to make a small 2-cm horizontal cut. Resorbable Vicryl sutures were used to clamp the horn beneath the ovary and each ovary was removed using forceps and scissors. The uterine horns were then placed back into the body and the peritoneal cavity was closed using interrupted nylon sutures.

**ASSESSMENT OF ATHEROSCLEROTIC LESIONS IN THE AORTA AND AORTIC SINUS.** The aortas were dissected and the adherent (adventitial) fat was gently removed. Whole aortas were opened longitudinally from the aortic arch to the iliac bifurcation, mounted en face, and stained for lipids with oil red O. Hearts were embedded in optimum cutting temperature compound (OCT) (Tissue-Tek, Sakura, Torrance, California) and serial 7-μm-thick cryosections from the aortic sinus were captured using the BZ-X710 microscope (Keyence, Itasca, Illinois) and analyzed by BZ software using a BZ-X710 microscope (Keyence). Image analysis was performed by a trained observer blinded to the genotype of the mice. Lesion areas were quantified with image analysis software using a BZ-X710 microscope (Keyence). The lesion area in the aorta en face preparations was expressed as a percent of the aortic surface area, as previously reported (44). Necrotic core was measured by hematoxylin-eosin staining and quantified with image analysis software.

**MESO SCALE DISCOVERY.** IL-1β in mouse plasma samples was measured using the U-PLEX Mouse IL-1β Assay (Meso Scale Diagnostics, Rockville, Maryland) per the manufacturer’s instructions. The samples were read and analyzed by Meso Scale Discovery QuickPlex SQ120 instrumentation and Workbench 4.0 Software (Meso Scale Diagnostics).

**CYTOKINE ASSAY.** IL-18 in mouse plasma samples was measured using enzyme-linked immunosorbent assay kits for murine IL-18 (Abcam, Cambridge, Massachusetts). Cell culture supernatants were assayed using commercially available enzyme-linked immunosorbent assay kits for murine IL-1β and tumor necrosis factor-α (eBioscience, San Diego, California) according to the manufacturer’s instructions.

**IMMUNOFLOUORESCENCE STAINING AND IMAGE ACQUISITION.** For immunohistochemical staining of frozen sections, fixing and antigen blocking were performed using immunoglobulin from the species of the secondary antibodies. Next, the sections were incubated with primary antibodies overnight at 4°C, followed by incubation with the appropriate secondary antibodies conjugated with fluorescent dyes. For assessment of macrophage content, cells were detected using anti-MOMA-2 antibody and for colocalization staining, nuclei were counterstained with DAPI. Caspase-1 activity was detected by fluorescent labeled inhibitors of caspases (FLICA) staining. Images (3 sections per animal) were captured using the BZ-9000 microscope (Keyence) and analyzed by BZ analyzer software.

**SERUM LIPID PROFILES.** Mice were sacrificed and sera from mice were obtained at the end of experiments and after an overnight fast. Total cholesterol concentrations and lipid profiles were determined in duplicate by using a colorimetric assay (infinity cholesterol reagent, Sigma Diagnostics, St. Louis, Missouri) as described earlier (43).

**STATISTICAL ANALYSIS.** Results are reported as mean ± SEM. All data were analyzed with the GraphPad Prism statistical software version 7.
FIGURE 1 NLRP3 Deficiency Reduces Diet-Induced Atherosclerosis Development in Female But Not Male Mice

(A) Representative oil red O staining of aortic sinus plaque in Ldlr−/− and Nlrp3−/−Ldlr−/− mice (n = 12). (B) Quantification of area of aortic sinus plaques. (C) Quantification of lipid content of aortic sinus. (D) Representative oil red O staining of aortic en face Ldlr−/− and Nlrp3−/−Ldlr−/− mice (n = 12). (E) Quantification of aortic lesion coverage. Data are presented as mean value ± standard error of the mean. Statistical significance was determined using Student’s t-test.
FIGURE 2  Comparison of Necrotic Core and Macrophage Content in Diet-Induced Atherosclerosis Lesion of Female Versus Male Mice

A

\[ Ldlr^{-/-} \quad Nlrp3^{-/-} \]

\[ Ldlr^{-/-} \quad Ldlr^{-/-} \]

B

\[ Ldlr^{-/-} \quad Nlrp3^{-/-} \]

\[ Ldlr^{-/-} \quad Ldlr^{-/-} \]

C

\[ Ldlr^{-/-} \quad Nlrp3^{-/-} \]

\[ Ldlr^{-/-} \quad Ldlr^{-/-} \]

D

\[ Ldlr^{-/-} \quad Nlrp3^{-/-} \]

\[ Ldlr^{-/-} \quad Ldlr^{-/-} \]

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Statistical differences between 2 groups were assessed using a 2-sided Student’s t-test. Values of \( p < 0.05 \) were considered significant.

**SAMPLE SIZE AND POWER CALCULATIONS.** We followed the American Heart Association scientific statement on the recommendation of design and execution and reporting of animal atherosclerosis.
studies as published in 2017 by the American Heart Association Council on Arteriosclerosis, Thrombosis and Vascular Biology and Council on Basic Cardiovascular Sciences (45). The sample sizes needed in each experimental group were based on 80% power and 2-sided tests for 5% level of significance. Based on our prior experience, given expected experimental variance within a treatment group of up to 20%, we calculated that a minimum of 8 to 10 animals per group was required. To account for any additional variability or mortality during the experiments, we calculated a priori that 10 to 12 mice were used in each group. Both male and female mice were used and analyzed separately because this was the main focus of this study.

RESULTS

NLRP3 INFLAMMASOME PLAYS A GREATER ROLE IN HFD-INDUCED ATHEROSCLEROSIS IN FEMALE COMPARED WITH MALE LDLR-/− MICE. To assess sex differences in inflammasome-mediated acceleration of atherosclerosis, we generated Nlrp3−/−Ldlr−/− mice and fed them an HFD for 12 weeks. Despite similar blood cholesterol levels and lipid profile (Table 1), in female mice, NLRP3 deficiency resulted in a 30% decrease in plaque size in the aortic root compared with Ldlr−/− alone, whereas this difference was not significant in males (Figures 1A and 1B). DKO females also exhibited 32% less lipid content in aortic root plaque (Figure 1C) and 38% less lipid coverage in aortic en face compared with control animals, whereas in males there was no significant difference between DKO and control animals (Figures 1D and 1E). In HFD-fed male mice, NLRP3 deficiency did not affect necrotic core size (Figure 2A) or macrophage content (Figure 2C), whereas these parameters were significantly reduced in NLRP3-deficient females compared with control animals (Figures 2B and 2D).

Lipid accumulation and cell death within the lesions contribute to activation of inflammatory cells that release proinflammatory and proatherogenic mediators into the serum (46,47). We measured IL-1β and IL-18 in the serum and found significantly higher concentrations of IL-1β and IL-18 in female Ldlr−/− mice compared with male mice (Figures 3A and 3B). Additionally, peritoneal macrophages were isolated from female and male Ldlr−/− mice after 12 weeks HFD. Cells were pretreated with lipopolysaccharide and then stimulated with NLRP3 activator ATP. Female macrophages secreted significantly more IL-1β, but not tumor necrosis factor-α, compared with male macrophages (Figures 3C and 3D).

NLRP3 ACTION IN HEMATOPOIETIC CELLS MODULATES ATHEROSCLEROSIS IN FEMALES. The previously discussed results suggest that the NLRP3 inflammasome plays a greater role in lesion development in female mice compared with male mice. We next used a BM chimera (donor → irradiated recipient: wild-type→Ldlr−/−; Nlrp3−/−→Ldlr−/−) approach to determine the role of NLRP3 in BM-derived cells in the sex difference in inflammasome-mediated acceleration of atherosclerosis. After 12 weeks on HFD, despite similar blood cholesterol levels and lipid profile (Table 2), female recipients of Nlrp3−/− BM developed significantly smaller lesions in the aortic sinus and lower lipid content in aortic root (Figures 4A to 4C) and less aorta en face lipid coverage (Figures 4D and 4E) than did female recipients of wild-type BM. However, this protection was not observed in males (Figures 4A to 4E). These data confirmed our previous results and further suggest that the NLRP3 inflammasome in hematopoietic cells plays a greater role in HFD-mediated atherosclerosis in females than in males in the Ldlr−/− model.

SEX HORMONES MODULATE NLRP3-MEDIATED ATHEROSCLEROSIS. To determine whether the differences we observed between sexes were mediated by sex hormones, we performed CAS in male Ldlr−/− and Nlrp3−/−Ldlr−/− mice; sham-surgery in male Ldlr−/− and Nlrp3−/−Ldlr−/− mice were done to control and rule out any effects that may be caused by the surgery itself (48). After 1 week of recovery, all mice were fed HFD for 12 weeks before sacrifice. As expected based on our previous data, in sham-operated male mice, there was no difference in aortic lesion size between genotypes (Supplemental Figure 1A), but in the CAS group, DKO mice now showed significant protection (Figure 5A). Similar protection in CAS DKO mice was observed in terms of lipid content in the aortic sinus (Figure 5B), and in aortic en face (Figure 5C), but not in sham-operated male DKO mice.

| TABLE 2 Total Cholesterol Level, Lipoprotein Profile and Triglyceride Concentrations in Serum (mg/dl) of Mice |
|-----------------|-------------------------------|---------|----------|-----------|----------|
| Donor (BM) to Recipient | TC | HDL | LDL | TG |
| WT to Ldlr−/− M | 987±78 | 59±15.7 | 110±19.2 | 134±28 |
| Nlrp3−/− to Ldlr−/− M | 901±48 | 62±12.5 | 104±21 | 128±20.1 |
| WT to Ldlr−/− F | 1,012±77 | 52±15.3 | 112±17.8 | 150±13.2 |
| Nlrp3−/− to Ldlr−/− F | 978±81 | 61±17.4 | 117±13.9 | 136±16.4 |

Values are mean ± SEM.  
BM = bone marrow; WT = wild-type; other abbreviations as in Table 1.
FIGURE 4 Nlrp3 Deficiency in Bone Marrow Cells Reduces Diet-Induced Atherosclerosis Development in Female But Not Male Mice

All mice were on Ldlr⁻/⁻ background. Irradiated Ldlr⁻/⁻ mice received wild-type or Nlrp3⁻/⁻ bone marrow cells. After 8 weeks reconstitution, the mice were fed high-fat diet for 12 weeks. (A) Representative oil red O staining of aortic sinus plaque in mice (n = 12). (B) Quantification of area of aortic sinus plaques. (C) Quantification of lipid content of aortic sinus. (D) Representative oil red O staining of aortic en face (n = 12). (E) Quantification of aortic lesion coverage. Data are presented as mean ± SEM. Statistical significance was determined using Student’s t-test. WT = wild-type.
Comparison of Aortic Sinus Lesion Size in Nlrp3 Ldlr DKO in Male CAS and Female OVX Mice

Oil red O staining of aortic root (A), aortic root lipid content (B), and aortic en face (C) in male CAS mice ($n = 8$ to 11). Oil red O staining of aortic root (D), aortic root lipid content (E), and aortic en face (F) in female OVX mice ($n = 8$ to 10). Data are presented as mean ± SEM. Statistical significance was determined using Student's t-test. CAS = castration; OVX = ovariectomy.
female mice (Figures 6A and 6B). Interestingly, CAS significantly increased FLICA positivity in the aortic roots compared with sham-operated males (Figures 6C and 6D); however, OVX did not alter FLICA positivity in aortic roots compared with sham-operated females (Supplemental Figure 2). These findings indicate that at baseline, female mice have more NLRP3 inflammasome activation in plaque macrophages than males, which may drive the enhanced macrophage accumulation. Additionally, in agreement with the effect on plaque formation, loss of testosterone caused by CAS exacerbates inflammasome activity.

**DISCUSSION**

Inflammation plays an important role in atherogenesis, plaque rupture, and subsequent thrombosis leading to acute ischemic syndromes (13). Recent studies have suggested key roles for the proinflammatory cytokines IL-1β and IL-1α in atherosclerosis (46,50-53). Notably, genetic deficiency of IL-1α, even when restricted to BM-derived cells, mitigates atherosclerotic burden in a mouse model (52), and this protective effect is even more pronounced when combined with depletion of IL-1β (52).

The NLRP3 inflammasome is a multicomponent complex that tightly regulates the maturation and secretion of IL-1β and IL-1α in atherosclerosis (46,50-53). Given that the NLRP3 inflammasome regulates multiple cytokines, some researchers have suggested that targeting NLRP3 or caspase-1 may yield better outcomes in inflammatory disease than targeting the IL-1β alone or other IL-1 cytokines in isolation (16). In this study, we found that Nlrp3 deficiency decreased lesion development and aortic lipid accumulation in HFD-fed Ldlr+/− female mice, but although the trend was evident in male mice, this protection was not significant, suggesting that female mice may have greater sensitivity to NLRP3 inflammasome compared with male mice, whereas in both genders NLRP3 plays a role. Furthermore, we showed that this protection was related to inflammasome activity in hematopoietic cells, because BM chimera females that received NLRP3-deficient BM showed a similar reduction in atherosclerosis. Interestingly, this difference was lost on OVX, suggesting a role for estrogen and/or progesterone in the effect. In contrast, in male Ldlr-deficient mice, CAS conferred significant protection from lesion development and lipid accumulation. Taken together, these data suggest that sex hormones play a role in inflammasome-mediated atherogenesis and thus may influence the response to inhibitors of IL-1β, IL-1α, and IL-18.
A role for sex in modulating atherogenesis is supported by early work in mouse models, which demonstrated that atherosclerotic lesions in the aortic root were larger in female mice (56-58), although this observation has not been a consistent finding across studies (59). The general understanding of how sex hormones influence the immune system is that estrogens have immune-enhancing effects, whereas progestosterone and androgens, such as testosterone and dihydrotestosterone, exert mainly immunosuppressive effects (60). Consistent with this paradigm, estrogen markedly enhances lipopolysaccharide-induced IL-1β promoter activity in the murine macrophage RAW cell line (28,29), and macrophages from female rats secrete more IL-1β and IL-6 in response to lipopolysaccharide (28). Females typically develop a more vigorous innate and adaptive immune response to antigen challenges (61,62), which can accelerate pathogen clearance but can also lead to increased immune-related pathology, such as autoimmune or inflammatory diseases (12,63). Androgens exert an overall inhibitory effect on Th1 differentiation (64) and suppress inflammatory immune cells, such as dendritic cells and macrophages (65). Of interest, CAS signaling on the NLRP3 in dendritic cells and macrophages (65) demonstrate, revealing complexities that challenge context-dependent. For example, in hepatocellular and endometrial carcinoma cells, estrogen upregulates the NLRP3 inflammasome via ERβ (39,67). But in the brain and in fibroblast-like synoviocytes, estrogen inhibits activation of the NLRP3 inflammasome (22). ERα and ERβ are NOD-like receptors (NLRs) transcription regulation factors, because they both regulate NLR expression and promote inflammasome colocalization, and a selective ERα antagonist significantly inhibits NLRP3 expression and inflammasome activity (68). Furthermore, the role of inflammasome activation in inflammatory pathways may also be context-specific, because in contrast to our findings, gene expression studies suggest that the inflammasome plays a more central role in abdominal aortic aneurysms in males than in females (69). Our finding of a role for female sex hormones in driving inflammasome-mediated atherogenesis in mice could be interpreted as contradictory to clinical studies, because menopause (a state of estrogen deficiency) is associated with higher ACVD risk in human females. However, the effect of estrogen deficiency on inflammasome activity in human subjects is unknown, and the enhanced risk of ACVD in post-menopausal females may also be related to advancing age and age-related alterations in inflammatory responses that are independent of inflammasome activity.

Despite the well-established link between inflammation and atherosclerosis, clinical data demonstrating a direct benefit of targeting inflammation had been absent until the CANTOS trial showed the potential for using anti-inflammatory therapy (anti-IL-1β), confirming that IL1β is an important potential therapeutic target for human atherosclerosis and related complications (40). However, for this approach to be clinically useful, it is critical to identify subsets of patients who will derive maximum benefits from canakinumab (or other anti-inflammatory agents) and are at low risk for serious infection. In the CANTOS trial, risk reduction with anti-IL1β therapy was observed in both men and women; however, women formed only 26% of the cohort, indicating that women may be more responsive than men to this therapy (41). Furthermore, the fact that males in the CANTOS trial demonstrated benefit from the anti-IL1β therapy does conflict with the findings reported here, because NLRP3 inhibition could affect several pathways beyond IL-1β, including IL-1α and IL-18 as stated previously. Indeed, there are still important gaps in the understanding of the role of the NLRP3 inflammasome and caspase-1 in
FIGURE 6 Comparison of Active Caspase-1 Macrophages in Diet-Induced Atherosclerosis Lesion

(A) Representative images for caspase-1 positivity in lesion macrophages of male versus female Ldlr<–/– mice. Caspase-1 activity was assessed by fluorescent-labeled inhibitors of caspases (green) in macrophages (MOMA-2) (red) in atherosclerotic lesions of Ldlr<–/– mice fed high-fat diet for 12 weeks. (B) Quantification of active caspase-1<sup>+</sup> cells in lesion macrophages (n = 10). (C) Representative images for caspase-1 positivity in lesion macrophages of male sham versus CAS Ldlr<–/– mice (n = 9 to 11). (D) Quantification of active caspase-1<sup>+</sup> cells in lesion macrophages. Data are presented as mean value ± standard error of the mean. Statistical significance was determined using Student’s t-test. FLICA = fluorescent labeled inhibitors of caspases; other abbreviation as in Figure 5.
atherosclerosis. Of interest, a recent study linked increased NLRP3 expression in human carotid plaques to pathological features, such as vascular inflammation, plaque composition, and vulnerability (16). The authors highlighted the importance of NLRP3 inflammasome and caspase-1-driven IL-1β and IL-1α production in atherosclerotic carotid plaques, supporting the view that release of both of these IL-1 isoforms is determined by the NLRP3-caspase-1 pathway in atherosclerotic plaques (16).

A novel, common, and powerful cardiovascular risk factor has recently emerged: clonal hematopoiesis of indeterminate potential, which arises from somatic mutations in hematopoietic stem cells (17). Studies have shown that individuals who acquire somatic clonal hematopoiesis of indeterminate potential mutations with age have a 40% increase in cardiovascular risk, independent of traditional risk factors (70). Most cases of clonal hematopoiesis of indeterminate potential are caused by mutations in only a handful of genes, including TET2 (17,70-72). Ldlr-/- mice engineered to bear the TET2 loss of function that is similar to clonal hematopoiesis and increased cardiovascular disease risk in humans (17) had activated NLRP3 inflammasome in myeloid cells, enhanced IL-1β production, and developed accelerated atherosclerosis (73,74). Even though male-to-female mice comparisons were not reported, it is of interest that, in both of these experimental studies the recipient mice that developed increased NLRP3-induced accelerated atherosclerosis were female (73,74).

Women have higher death rates following myocardial infarction than men (75,76), because of differences in the pathogenesis of atherosclerosis, differential efficacy of drugs (77,78), and because vascular assist devices may fit men better than women (79,80). As Clayton and Tannenbaum have argued (81), failure to analyze for male and female clinical trial participants separately may mask important differences in the effects of interventions, toxicity, symptoms, or adverse effects. To date, most clinical studies in this area contain a majority of male subjects, and no trial that we are aware of pre-specified analysis of differences by sex. Therefore, understanding how the immune response differs in men and women in the context of atherosclerosis may improve the treatment of cardiovascular disease.

In summary, there is a need for additional investigation on the role of estrogens, progesterone, and testosterone on the NLRP3 inflammasome and IL-1β as well as IL-1α signaling in atherogenesis as it relates to the biologic mechanisms underlying pathophysiological processes in males and females. Our data add weight and a sense of urgency to the efforts of Robinet et al. and the Council on Arteriosclerosis, Thrombosis and Vascular Biology to encourage preclinical arterial pathology researchers to consider sex as a biologic variable when designing and reporting experiments (1), which will improve the design of clinical trials, and help optimize cardiovascular care for men and women.

CONCLUSIONS

The present study suggests that loss of NLRP3 inflammasome components leads to more significant reductions in atherosclerotic plaque size and lipid content in female mice than in male mice. Furthermore, CAS increases dependency on NLRP3 inflammasome components for atherogenesis, and increases inflammasome activity, suggesting that testosterone plays an inhibitory role, blocking inflammation in atherogenesis. OVX reduces dependency of atherogenesis on NLRP3 inflammasome components, suggesting that female sex hormones sensitize inflammation in atherogenesis. Our data provide biologic insights into the clinical merit of anti-NLRP3-directed therapies, and the biologic mechanisms underlying pathophysiological processes in males versus females as they pertain to atherosclerosis and the NLRP3 inflammasome, which could help inform the design of future clinical trials.

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COMPETENCY IN MEDICAL KNOWLEDGE: The CANTOS trial suggested that IL-1β-directed therapy with a neutralizing monoclonal antibody moderately reduces recurrent ischemic events and cardiovascular death among patients with coronary artery disease and elevated C-reactive protein. Therefore, IL1β, which is under the control of the NLRP3 inflammasome and caspase-1, is an important potential therapeutic target for human atherosclerosis and related complications. However, NLRP3 inflammasome also controls secretion of IL-1α and IL-18, inflammatory cytokines that have also been implicated in development of atherosclerosis, and some researchers have suggested that targeting NLRP3-caspase-1 may yield better outcomes than targeting the IL-1β alone or IL-1 isoforms in isolation. Herein, we show that sex hormones may be involved in NLRP3 inflammasome-mediated atherogenesis and may lead to differential responses to anti-NLRP3 therapy between males and females. In a mouse model of atherosclerosis, females with global Nlrp3 deletion or those receiving Nlrp3−/− BM developed significantly fewer lesions in the aortic sinus and decreased lipid content in aorta, but Nlrp3 deficiency did not confer similar protection in males. Ovariectomized female mice lost protection mediated by NLRP3 deficiency, whereas castrated males showed stronger correlations between NLRP3 inflammasome and atherosclerosis. Overall, the findings of present study suggest that testosterone may play an inhibitory role by blocking NLRP3 inflammasome and inflammation in atherogenesis, whereas female sex hormones may promote NLRP3 inflammasome-mediated atherosclerosis.

TRANSLATIONAL OUTLOOK: The specific role and underlying mechanisms of inflammasome activation and inflammation in atherogenesis are topics of active research. The role of the NLRP3 inflammasome pathway in diet-induced atherosclerosis is still controversial, and the impact of sex hormones has not been explored. In this study we observed sex-specific effects of the NLRP3 inflammasome on atherogenesis in LDLR-deficient mice, with NLRP3 inflammasome playing a more prominent role in atherosclerosis in female mice than in males. The CANTOS study demonstrated modest therapeutic benefit of a monoclonal antibody targeting IL-1β (canakinumab) in male and female patients with previous myocardial infarction, indicating that IL-1β is an important therapeutic target. However, the NLRP3 inflammasome controls not only IL-1β secretion, but also IL-1α and IL-18, leading some researchers to advocate that targeting NLRP3-caspase-1 may yield better outcomes. Furthermore, a secondary analysis of the CANTOS trial revealed that whereas women and men showed similar clinical efficacy with canakinumab, only 26% of the participants were female, suggesting that a smaller sample size was needed for females to achieve the same clinical benefit. Therefore, finding ways to identify subsets of patients who will derive maximum benefits from canakinumab (or other anti-inflammatory agents) is crucial, and it is critically important to understand the role of sex in NLRP3 inflammasome-mediated IL-1β and IL-1α-driven inflammation in atherosclerosis. The present study lends support to the impact of estrogens and testosterone on the inflammasome in atherogenesis and yields important information on biologic mechanisms underlying pathophysiological processes in atherosclerosis in both sexes. The results of the present study may help design future clinical trials, with the objective to personalize cardiovascular care for men and women.

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APPENDIX For supplemental figures, please see the online version of this paper.