

Female rats demonstrate increased acute immune response to *Staphylococcus aureus* periprosthetic implant infection compared to males.

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INTRODUCTION: Sex is an important variable in immunity and therefore in infectious processes across medicine (1). It has been shown that biological females mount a more robust immune response against viruses and therefore many viral diseases are less common in females (1). Similarly, it is becoming increasingly evident that males are more disposed to peri-prosthetic joint infections following hip and knee total joint arthroplasty than females (2). The reason behind these sex differences in immune response are less clear. The difference in X and Y chromosome complements possessed by males and females may contribute to the differences as many X-linked genes encode protein with immune-related functions. Sex hormones, such as androgens and estrogen, may contribute to the disparity in immune response as well with these hormones affecting autoimmunity, T cell tolerance and B cell numbers (1). Additionally stimulated female T cells have been shown to upregulate more proinflammatory cytokines than male T cells (3). *Staphylococcus aureus* (*S. aureus*) is the most common cause of periprosthetic infection and male sex is associated with higher risk of nasal colonization and infection with this organism (4). The objective of this study was to test the hypothesis that female rats would demonstrate a stronger immune response in the face of an implant associated *S. aureus* infection compared to male rats.

METHODS: *Animals and surgical procedures:* With IACUC approval, female and male Sprague-Dawley rats were anesthetized and a craniolateral incision between the vastus lateralis and the biceps femoris was created to access the femur (index surgery, Day 0). An Accupen (RISystem) was used to place two 1.7mm titanium screws at the proximal and distal ends of the femur, and to create a mid-diaphyseal empty drill hole to expose the medullary cavity. A collagen sponge impregnated with 250 μ L *S. aureus* ATCC 25923 at 1×10^5 CFU/250 μ L (INF) or saline (CON) was positioned over the screws and the empty hole. The rats were treated with perioperative analgesics (extended-release buprenorphine 0.3 mg/kg q72hrs SC and meloxicam 2 mg/kg q24hrs SC). Seven days later (Day 7) the rats were humanely euthanized and the hardware and tissue surrounding the incision were aseptically collected for microbial analysis, histology and RNA and protein isolation. *Microbial Analysis:* The tissue was vortexed for 30 secs in 5 mL sterile phosphate buffered saline which was used for colony counts. The hardware was washed in 1 mL PBS 3x to remove planktonic bacteria, then placed in 0.3% Tween20 for 5 minutes of sonication. Bacterial counts from the hardware and muscle were determined after serial dilution and plating on Petrifilms. *Histology:* Tissue was fixed in 4% paraformaldehyde (PFA) (Sigma) for >24 before being embedded in paraffin blocks. 5 μ m sections were stained with H&E, trichrome (fibrosis), toluidine blue (mast cells), and Brown and Hopps (bacteria) was performed, followed by image analysis (ImagePro Plus software, MediaCybernetics). *RT-qPCR:* The muscle tissue caudal to the infection site was homogenized, the RNA was isolated, and the cDNA extracted for analysis. qPCR for the following cytokines was performed in triplicate: IL-6, IL-1 β , NOS2, IL-10, IL-17, TGF- β , and SOCS3.

RESULTS SECTION: Histologically, the female infected rats demonstrated significantly higher total immune cell counts than infected male rats as seen on the Toluidine Blue histological images of the skin surrounding the surgical site. All infected rats demonstrated significant upregulation of IL-1 β , NOS2, SOCS3, and IL-17 compared to all sham-operated rats. Infected female rats showed significant upregulation of IL-6, IL-17, and SOCS3 compared to non-infected female rats. This trend was not seen between the infected and control male rats. *Sample Size:* n=3 male INF, n=3 female INF, n=6 male CON, n=6 female CON. *Statistics:* Statistical analyses were done using GraphPad Prism 8 statistical software. Depending on the nature of the data, a paired or unpaired t-test, one-way or two-way ANOVA was performed.

DISCUSSION: These results suggest that in the face of similar burdens of *S. aureus* periprosthetic infection females mount a more robust immune response as shown by the upregulation of proinflammatory cytokines IL-6, IL-17, and SOCS3 and the significantly increased total immune cell infiltration of the surgical area compared to males. These findings support the globally held impression that men are more at risk for periprosthetic infection than women. Moreover, while the acute response to excessive inflammation is widely appreciated as a unifying component in many chronic diseases including infection and estrogen has been implicated to play a protective role against excessive inflammation, the exact target and molecular pathways that underlie the beneficial effects of female sex in humans has remained uncertain. The process of inflammation resolution is an active process and initiated by the recruited cells themselves. It is further mediated by a switch in the local production of lipid mediators from the proinflammatory prostaglandins and leukotrienes to the pro-resolving and tissue-reparative mediators, including the lipoxins and resolvins (6). Using targeted lipid mediator profiling in an investigation of sex-dependent accelerated resolution of inflammation between a cohort of men and women, Rathod showed that the balance of pro-resolving to pro-inflammatory lipid mediators was tipped in favor of resolution in females. The arachidonic acid cascade is a metabolic pathway that produces various bioactive lipids called eicosanoids, which are involved in inflammation and immune responses. SOCS3 modulates the arachidonic acid cascade by affecting the expression and activity of enzymes that synthesize eicosanoids, such as D-resolvin. However, the modulation of D-resolvin by SOCS3 is dynamic and context-dependent that influences the balance between inflammation and resolution. Interestingly control males produce more SOCS3 than control females which may contribute to their decreased immune response (7). Further studies exploring the regulation of pro-resolving mediator on inflammatory cells and the impact of inhibiting these mediators on the resolution of inflammatory responses are important to confirm the sexual dimorphism. A notable limitation of this study is the sample size of n=3 for the infected groups. More infected animals would contribute to making this a more powerful study.

SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): *S. aureus* infections of orthopedic implants is a major problem being faced by orthopedic surgeons across specialties, therefore understanding the immune response and interaction between the host and the microbial invader is of tantamount importance in developing new treatment strategies. The dramatic disparity in infection rates between males and females emphasizes the importance of individually targeted treatment plans when fighting infection as both the baseline immune status and the immune response will be different depending on a patient's biological sex.

REFERENCES: (1) Wilkinson *et al* 2022. (2) Choong *et al* 2020. (3) Hewagama *et al* 2009. (4) Stenson *et al* 2022. (5) Serhan 2014. (6) Rathod *et al* 2016. (7) Qin *et al* 2012.