# CXCR2 perturbation promotes *Staphylococcus aureus* implant-associated infection

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MICROBIOLOGY, IMMUNOLOGY, AND PATHOLOGY

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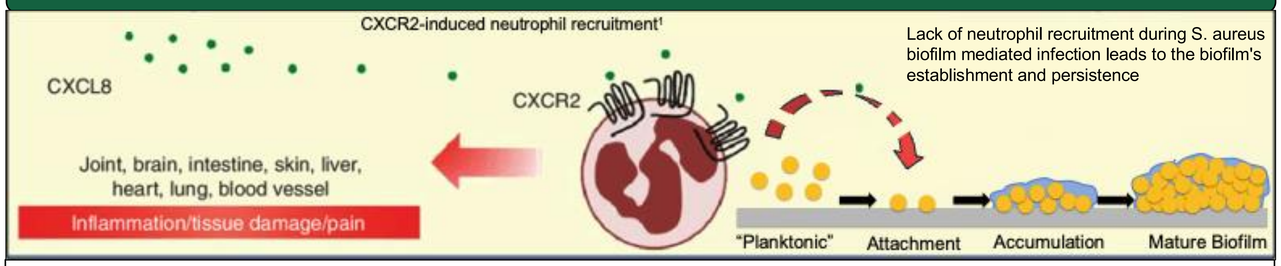
## Abstract

Staphylococcus aureus is a leading cause of implant-associated infections due to biofilm formation, antimicrobial resistance, and immune modulation, resulting in persistent infection and bacteremia. Neutrophil recruitment via CXC chemokine receptor 2 (CXCR2) is critical for host defense, but its role in biofilm-associated infection remains unclear. We investigated CXCR2 function in a murine S. aureus implant infection model using subcutaneous catheters infected with a community-associated MRSA strain. Mice received Lipocalin-2 (Lcn2) to enhance CXCR2 expression or AZD5069 to inhibit CXCR2 signaling. Both Lcn2 and AZD5069 treatment resulted in significantly increased bacterial burdens in implants and surrounding tissues, accompanied by elevated Cxcr2 expression. These findings demonstrate that disruption of CXCR2 signaling, either upregulation or inhibition, impairs bacterial control, suggesting that CXCR2-mediated neutrophil responses require precise regulation during S. aureus biofilm infection.

## Overview

### CXCR2 interactions on neutrophils:




CXCR2 is a G-coupled protein receptor expressed on immune cells, notably neutrophils where it mediates recruitment to infection sites, chemotaxis, integrin activation for extravasation and mobilization of hematopoietic stem cells from the bone marrow.[2,3]

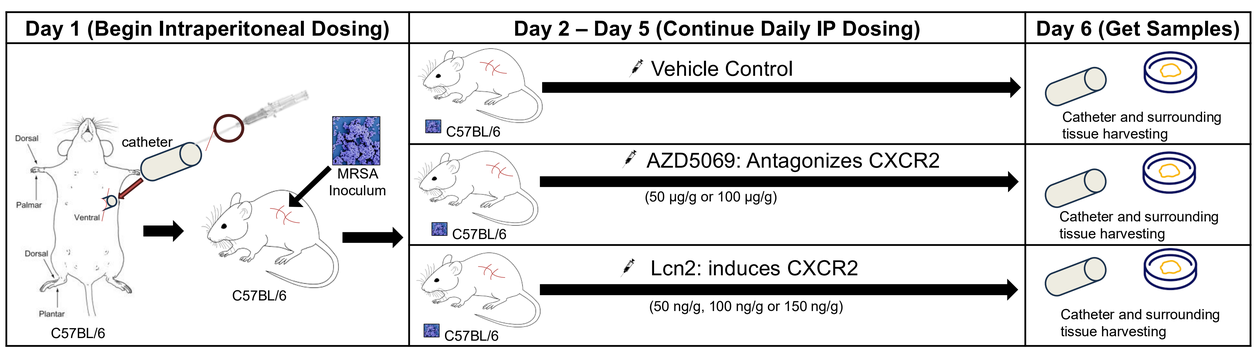
## Methods

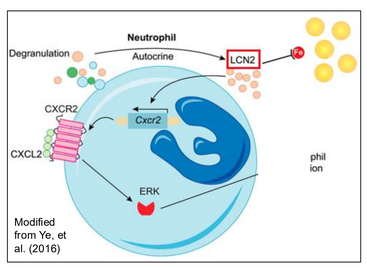
***S. aureus* strain and culture conditions:** *S. aureus culture:* USA300 MRSA grown in Brain Heart Infusion Broth (16 hr, 37 °C, 250 rpm), washed with PBS; and optical density 600 used for inoculum.

**Mouse flank catheter model of *S. aureus* infection:** 8-week-old C57BL/6 mice were anesthetized and subcutaneous catheters were surgically inserted to the left flank. 20 µL of MRSA USA300 ($10^3$ CFU) was subsequently injected intraluminally. Study duration: 5 days.

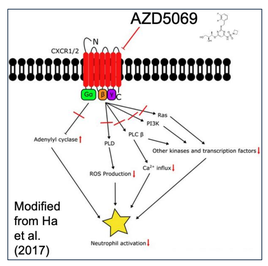
**-Treatment groups:** Daily IP injections of lipocalin-2 (Lcn2; 50, 75, 100 ng/g) or CXCR2 inhibitor AZD5069 (50 µg/g in 45% PW, 40% PEG-300, 10% DMSO, 5% Tween-80).

-**Control groups:** Control groups included infected mice receiving daily IP vehicle and sterile mice (no inoculum) receiving daily IP vehicle or treatment.




**Lcn2 as a CXCR2 Inducer**

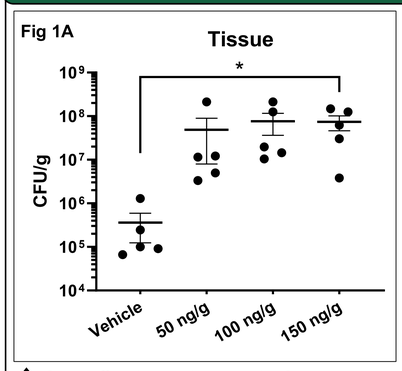


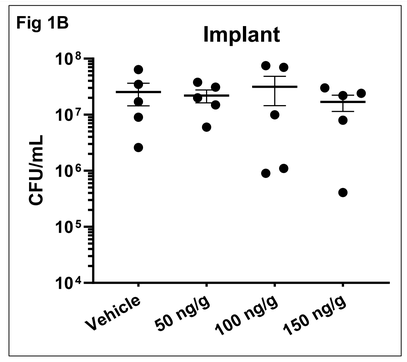

**AZD5069 as a CXCR2 Antagonist**

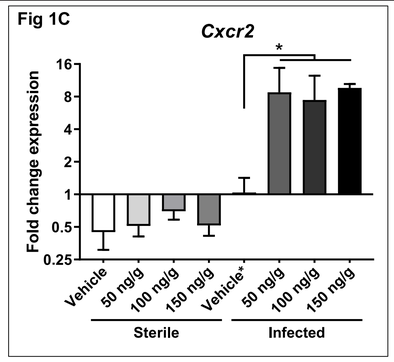
**Processing:** Mice were euthanized; catheters were removed, vortexed, and sonicated in PBS to dissociate bacteria. Surrounding tissue and spleen were collected. Tissue samples homogenized in PBS with protease inhibitors for quantitative real-time PCR. Bacterial burdens were quantified on blood agar plates and expressed as CFU/mL (catheters) or CFU/g (tissue).

## Results

### Lcn2 promotes spread of *S. aureus* from biofilm into soft tissue:

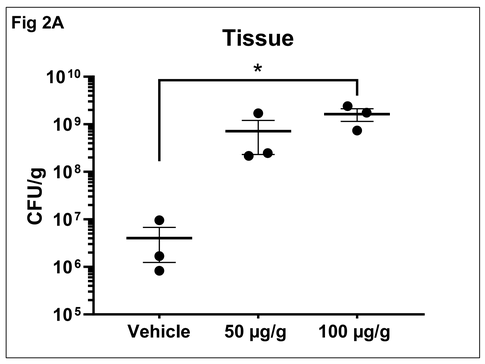


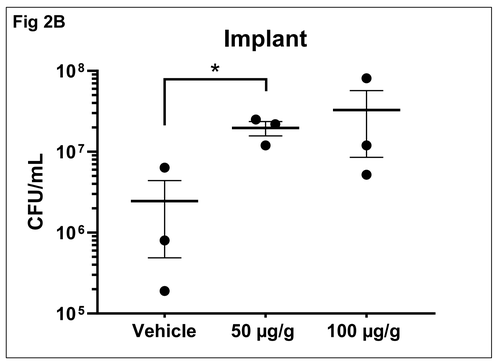



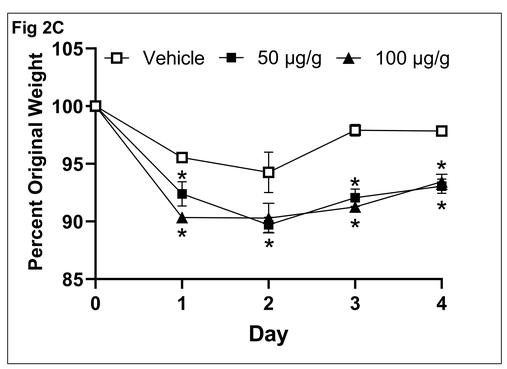



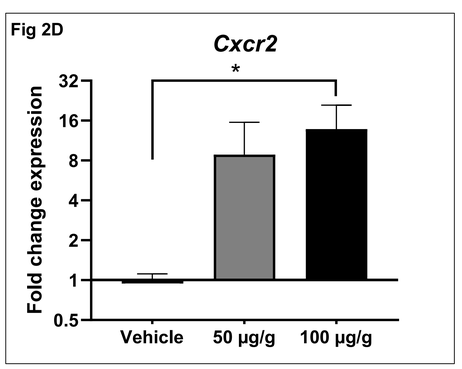

⬆ Lcn2 treatment increased bacterial burdens in catheter-associated tissues without affecting implant-associated burdens (**Fig 1A**, **B**). Infected tissues demonstrated a ~2-fold increase in Cxcr2 transcription versus sterile controls, while Lcn2 had no effect in sterile implants (**Fig 1C**), indicating an increased presence of CXCR2-expressing cells at active infection sites. Furthermore, *Cxcr2* expression in tissues associated with sterile implants was unchanged in mice receiving any dose of Lcn2, revealing that recruitment of CXCR2 expressing cells was depending on infection Lcn2 induced a dose-dependent ~8-fold increase in Cxcr2 expression in infected tissues only, with no changes in spleen. (data not shown). Together, *S. aureus* infection recruits CXCR2-expressing cells, and that Lcn2-mediated enhancement of *Cxcr2* induction promotes bacterial dissemination from the biofilm into surrounding soft tissues.

### AZD5069 promotes *S. aureus* biofilm infection development:




⬅ AZD5069 increased bacterial burdens in catheters and surrounding tissues and was associated with greater weight loss versus vehicle (**Fig 2A-C**). AZD5069 also induced an 8–14-fold increase in Cxcr2 expression (**Fig 2D).** The data demonstrate that inhibition of CXCR2 activity worsens S. aureus infection severity, promoting higher bacterial burden and tissue invasion.

## Conclusions

CXCR2 tightly regulates severity of *S. aureus* implant-associated infection; both upregulation and inhibition increase bacterial burden, indicating a requirement for balanced signaling. Dysregulated CXCR2 may promote infection via altered myeloid responses, including MDSC recruitment and macrophage-mediated immune modulation.

## References

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