Effect of Rheumatoid Arthritis on Periprosthetic Joint Infection

Active Rheumatoid Arthritis in a Mouse Model is not an Independent Risk Factor for Periprosthetic Joint Infection

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Ethics Statement Approval by ARC (Animal Research Committee) at the University of California, Los Angeles (UCLA)

The UCLA Chancellor's Animal Research Committee (ARC) approved all animal research submitted below (ARC# 2008-112-41) with regards to both forelimb/hindlimb murine surgery and research.

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Abstract

Introduction

Periprosthetic joint infection (PJI) represents a devastating complication of total joint arthroplasty associated with significant morbidity and mortality. Literature suggests a possible higher incidence of periprosthetic joint infection (PJI) in patients with rheumatoid arthritis (RA). There is, however, no consensus on this relative risk nor a well-defined mechanism of this purported risk. This study investigates how collagen-induced arthritis (CIA), a validated animal model of RA, impacts infectious burden in a well-established model of PJI.

Methods

Control mice were compared against CIA mice. Whole blood samples were collected to quantify systemic IgG levels via ELISA. *Ex vivo* respiratory burst function was measured via dihydrorhodamine assay. *Ex vivo* *Staphylococcus aureus* Xen36 burden was measured directly via colony forming unit (CFU) counts and crystal violet assay to assess biofilm formation. *In vivo*, surgical placement of a titanium implant through the knee joint and inoculation with *S. aureus* Xen36 was performed. Bacterial burden was then quantified by longitudinal bioluminescent imaging.

Results

Mice with CIA demonstrated significantly higher levels of systemic IgG compared with control mice ($p = 0.003$). *Ex vivo*, there was no significant difference in respiratory burst function ($p=0.89$) or *S. aureus* bacterial burden as measured by CFU counts ($p=0.91$) and crystal violet assay ($p=0.96$). *In vivo*, no significant difference in bacterial bioluminescence between groups was found at all postoperative time points. CFU counts of both the implant and the peri-implant tissue were not significantly different between groups ($p=0.82$ and 0.80 , respectively).

Conclusion

This study demonstrated no significant difference in *S. aureus* infectious burden between mice with CIA and control mice. These results suggest that untreated, active RA may not represent a significant intrinsic risk factor for PJI. Further mechanistic translational and clinical studies are, however, warranted to thoroughly investigate the infectious risk of RA.

Keywords: Periprosthetic joint infection, Rheumatoid arthritis, Collagen-induced arthritis, *Staphylococcus aureus*, Bioluminescence

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Introduction

Periprosthetic joint infection (PJI) is a challenging complication faced by orthopaedic surgeons and is the number one cause of implant failure following arthroplasty^{1,2}. PJI is associated with significant morbidity and mortality, with a higher 5-year mortality than both breast and prostate cancer³. Furthermore, the treatment of PJI places a tremendous economic burden on the United States healthcare system⁴⁻⁷. As the number of total joint replacements and subsequent PJIs are projected to increase in the United States^{4,8-11}, understanding factors that may alter infectious risk is crucial.

Rheumatoid arthritis (RA) is the most common autoimmune inflammatory arthropathy, affecting 0.5% of the general population¹²⁻¹⁴. RA is characterized by a dysregulated immune system, including a disordered innate immune response, an upregulated adaptive immune response against self-antigens, disordered cytokine activation, and dysregulated osteoclast and chondrocyte activation¹⁵⁻¹⁹. RA results in the autoimmune-based destruction of articular cartilage and often leads to debilitating joint pain ultimately necessitating arthroplasty^{16,20,21}. Some retrospective studies have demonstrated that patients with RA have a higher incidence of PJI than patients with osteoarthritis (OA) alone²¹⁻²⁷. Other studies, however, suggest that no such difference in infection rate exists between patients with RA and those with OA following total hip or knee arthroplasty²⁸⁻³⁰. The paucity of evidence as to the mechanistic etiology behind any purported increased infectious risk in patients with RA^{31,32}, along with the relative uncertainty of whether such an infectious risk truly exists, emphasizes the need for further research.

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85 As highlighted by the American Association of Hip and Knee Surgeons and the American College

86 of Rheumatology³¹, there is currently an unmet demand for thorough investigation of the

87 perioperative infectious risk associated with RA following arthroplasty. Perioperative

88 optimization of the host immune response is a crucial means by which to mitigate

89 postoperative infectious risk^{33,34}. While immunosuppressive medications used to treat RA

90 naturally carry risk for surgical site infection, it is unknown whether the immune dysregulation

91 of RA alone, independent of medications, carries infectious risk. Elucidating, isolating and

92 quantifying any purported infectious risk as well as further investigating its modifiability is

93 essential to formulating strategies to decrease the incidence of surgical site infection in this

94 patient population. Collagen-induced arthritis (CIA) is the gold standard animal model of RA and

95 forms the genetic and molecular background of the majority of genetically modified RA strains

96 in C57BL/6 mice. CIA is a clinically, radiographically, immunologically, and pathogenically well-

97 validated and reproducible model of RA³⁵⁻⁴¹. Given the multifactorial nature and challenges

98 associated with a clinical study of the RA patient population, this study aimed to interrogate a

99 well-validated mouse model⁴²⁻⁴⁵ of PJI as well as a well-validated model of RA³⁵⁻⁴¹ to determine

100 how RA alone effects perioperative infectious burden.

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Materials and Methods

Ethics Statement

All practices were set forth by federal regulations detailed in the Animal Welfare Act (AWA), PHS Policy for the Humane Care and Use of Laboratory Animals, the 1996 Guide for the Care and Use of Laboratory Animals as well as UCLA (University of California, Los Angeles) policies by procedures in the UCLA Animal Care and Use Training Manual. The UCLA Chancellor's Animal Research Committee (ARC) approved all animal research (ARC# 2008-112-41).

Bacterial Strain

Staphylococcus aureus Xen36 (PerkinElmer, Hopkinton, MA) is a derivative of a parent strain, ATCC-29525 (Wright), that contains a bioluminescent luxABCDE operon genomically integrated into a stable bacterial plasmid. When bacteria are metabolically active, a blue-green light with a maximal emission wavelength of 490nm is produced. This bioluminescent strain has been previously validated as the optimal strain with which to longitudinally monitor *S. aureus* burden in an implant-associated infection model⁴²⁻⁴⁵.

Bacteria was prepared according to recently published protocols⁴²⁻⁴⁷. Briefly, *S. aureus* Xen36 was first streaked onto tryptic soy broth (TSB) agar kanamycin plates (TSB plus 1.5% Bacto agar; BD Biosciences) and grown for approximately 24 hours at 37°C. Due to the presence of a kanamycin-resistant marker integral to the lux operon, this step removes potential contaminants. Single colonies were subsequently isolated and cultured in TSB with 200ug/mL kanamycin in a shaking incubator (196rpm) at 37°C for 16 hours (MaxQ 4,450, Thermo Fisher

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Scientific, Canoga Park, CA). An additional two-hour subculture was performed in order to obtain bacteria in mid-logarithmic phase growth. Following centrifugation, cells were pelleted, resuspended and washed in Phosphate-Buffered Saline (PBS). Bacteria was diluted with PBS to its final concentration of 1×10^3 *S. aureus* Xen36 CFUs/2 μ L, which was quantitated via spectrophotometry (OD, 600nm; Biomate 3; Thermo Fisher Scientific) and subsequently inoculated during the surgical procedure.

Induction of Collagen-Induced Arthritis

Eight week-old C57BL/6 wildtype mice (Jackson Laboratories, Bar Harbor, ME) were housed and stored with a 12-hour light and dark cycle with free access to water and a standard pellet diet. Veterinary staff monitored mice daily to verify their health throughout the duration of the study. A maximum of four mice were permitted to be housed in each cage. All mice were housed in the same room to minimize confounding.

The protocol for induction of CIA published by Inglis et al³⁶ was used on eight week old C57BL/5 mice. Briefly, a mixture of chicken type II collagen dissolved in 0.1M acetic acid was mixed with an equal volume of complete Freund's adjuvant (all reagents purchased from Sigma-Aldrich, Saint Louis, MO, USA). Eight week-old mice were then anesthetized using isoflurane (2%) and 0.1 mL of the resulting solution was injected at the base of the tail of each mouse. A booster injection was given two weeks following the primary immunization. Mice were randomized either to receive the immunization (CIA group) or sterile saline (control group). Mice were clinically evaluated six weeks after the primary immunization, as time to arthritis onset is

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between three and six weeks³⁶. The paws of each mice in the CIA group were given a score of 0, 1, 2, or 3 based on the amount of dorsal edema, with 0 correlating with no edema and 3 correlating with severe edema. A sum total score was generated for each mouse, with a maximum score of 12. As the incidence of arthritis after immunization is reportedly 50-75%³⁶, only mice that clinically showed any edema on the dorsum of their paws six week after induction were selected for *ex vivo* and *in vivo* experiments. All mice were fourteen weeks of age at the time of experiments.

Ex Vivo Mouse IgG ELISA to Confirm Systemic Effect of CIA

In order to confirm an altered immunologic profile in CIA mice, a mouse IgG ELISA (Bethyl Laboratories, Montgomery, TX) was performed to quantify systemic IgG levels. Briefly, whole blood was collected from six mice in the CIA group and six mice in the control group via cardiac puncture under 2% isoflurane inhalation anesthesia. Ethylenediaminetetraacetic acid (EDTA) was added to blood samples in a 1:10 ratio to prevent coagulation. 100 µL of blood from each mouse was added to mouse IgG pre-coated wells and mixed with 100 µL of anti-IgG Detection Antibody. Streptavidin-conjugated horseradish peroxidase was then added followed by 3,3',5,5'-tetramethylbenzidine. The colorimetric reaction was then terminated with 100 µL of 0.3 M sulfuric acid and the absorbance of the yellow resultant product was measured for each sample at 450nm using a plate reader (FLUOstar Omega, BMG Labtech, Ortenberg Germany). Per Bethyl Laboratories protocol, a four-parameter curve was subsequently generated using standardized concentrations of IgG. This curve was interpolated to allow for quantitation of IgG concentration in the CIA mice blood samples as well as control samples.

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168 *Ex vivo Quantification of Respiratory Burst*

169 Blood was collected as above from six mice in each experimental group. 100 μ L of blood was
 170 added from each mouse to each well within a 96-well flat bottom plate (Corning Costar,
 171 Corning, New York). A dihydrorhodamine (DHR) 123 assay was used to assess reactive oxygen
 172 species (ROS). Briefly, 10 μ L of DHR 123 Assay Reagent, 25 μ L of Phorbol myristate acetate and
 173 two 2mL of Red Blood Cell Lysis Buffer was added to each plate. An excitation filter of 485nm
 174 and an emission filter of 520nm was used to quantitate mean fluorescent intensity using a
 175 fluorescent plate reader (FLUOstar Omega, BMG Labtech, Ortenberg, Germany).

176

177 *Ex vivo CFU Quantification of S. aureus After Interaction with Whole Blood*

178 Six mice in each group underwent whole blood collection as above. 10 μ L of 1×10^3 *S. aureus*
 179 Xen36 CFU/mL and 10 μ L of blood was gently vortexed and incubated for one hour at 37°C. 20 μ L
 180 of the resulting solution was subsequently spread onto a TSB agar plate (tryptic soy broth [TSB]
 181 plus 1.5% Bacto agar; BD Biosciences) and incubated for 16 hours at 37°C. CFUs were counted
 182 for each plate and expressed as CFUs/mL.

183

184 *Ex vivo Quantification of Biomass of Biofilm*

185 Six mice in each group underwent whole blood collection as above. 100 μ L of 1×10^7 *S. aureus*
 186 Xen36 CFU/mL and 100 μ L of blood from each mouse were mixed into each well of a 96-well
 187 flat bottom plate. Additionally, 200 μ L of saline was used as a standardized control. The plate
 188 was incubated for 24 hours at 37°C (MaxQ 4450; ThermoFisher Scientific) to permit sufficient

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biofilm formation. Wells were then washed with PBS three times to remove blood and non-adherent bacteria. In order to quantify mass of the residual biofilm, a well-validated crystal violet assay (Abcam, Cambridge, United Kingdom) was performed and absorbance was measured by OD at 595nm (FLUOstar Omega, BMG Labtech, Ortenberg Germany). Values were reported as absorbance units.

In Vivo Mouse Surgical Protocol

Twenty-two mice were randomized into the following groups: 2 sterile controls, 8 infected controls (non-immunized), and 12 CIA-immunized. Mice were anesthetized via isoflurane (2%) inhalation. Implant surgery and inoculation was performed as described in prior protocols⁴²⁻⁴⁵. Briefly, a medial parapatellar approach was used to displace the patella and expose the distal femur. After subsequent reaming of the femoral intramedullary canal with a 25-gauge followed by a 21-gauge needle, an orthopaedic-grade titanium Kirschner wire (0.8mm in diameter, 6mm in length; DePuy Synthes, Warsaw, IN) was inserted in a retrograde fashion with 1mm protruding into the joint space. The protruding wire was then inoculated with either 2μL of sterile saline (0.9% NaCl, sterile control group) or 2μL of 1×10^3 *S. aureus* Xen36 CFUs/mL of bacteria (infected control and CIA groups). 5-0 polyglycolic acid sutures were used to close the surgical site. Following the procedure, all mice underwent high resolution X-rays using the IVIS Lumina X5 (PerkinElmer, Waltham, MA) to ensure appropriate placement of the implant.

In vivo Longitudinal Monitoring of Bacterial Burden and CFU Quantification

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As previously described⁴²⁻⁴⁵, the IVIS Lumina X5 (PerkinElmer, Waltham, MA) was used to obtain bioluminescent images representative of infectious burden on postoperative days (POD) 0, 1, 3, 5, 7, 10, 14, 18, 21, 25 and 28. Data were quantified as total flux (photons per second per cm² per steradian [photons/s/cm²/sr]). Mice were sacrificed on POD 28 and bacterial CFU counts of the bacteria adherent to the implant as well as in the surrounding joint tissue were quantified. To isolate adherent bacteria, implants underwent sonication in 500 µL 0.3% Tween-80 (ThermoFisher Scientific) in TSB. To isolate bacteria in the surrounding joint tissue, tissue was homogenized in 1000 µL of PBS with 4 homogenizing beads (Pro200H Series homogenizer; Pro Scientific) and the implants underwent sonication in 500 µL 0.3% Tween-80 (ThermoFisher Scientific) in TSB. Samples from tissue and implants were then drop plated and incubated for 16 hours at 37°C. CFUs were counted and expressed as CFUs/mL.

Clinical CIA Severity Scoring

Mice in the CIA group were given a clinical score of 0-12 to describe severity of CIA, as described above. Scores were given by a single observer at all bioluminescent imaging POD time points. Scores were calculated by noting the amount of swelling on the dorsum of each paw and giving each paw a score of 0-3³⁶.

Statistical Analysis

Consistent with prior research utilizing this implant infection model, each experimental group contained at least 6 mice in order to sufficiently power the study⁴³. A linear mixed effects regression model was used to analyze bioluminescent and clinical CIA score data. Data were

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232 expressed as mean $\pm$ standard error of the mean (SEM). A Student's t-test (one or two-tailed
233 where indicated) was conducted, and significance was defined as a p-value <0.05 .

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Results

Ex Vivo Mouse IgG ELISA, Quantification of Respiratory Burst, CFU and Biofilm Biomass

Systemic IgG levels as measured by an IgG ELISA assay were significantly higher in the blood of CIA mice ($3.1 \times 10^2 \pm 2.6 \times 10^1$ ng/mL) as compared to unimmunized, control mice ($1.9 \times 10^2 \pm 8.6$ ng/mL; $p = 0.003$) (Figure 1).

Respiratory burst, as measured by mean fluorescent intensity from a DHR 123 assay, was not significantly different between CIA mice ($4.9 \times 10^4 \pm 2.6 \times 10^3$) and control mice ($4.9 \times 10^4 \pm 2.4 \times 10^3$; $p=0.89$) (Figure 2).

The mean CFU/mL of *S. aureus* Xen36 after interaction with whole blood in the CIA group ($2.6 \times 10^3 \pm 1.7 \times 10^2$ CFU/mL) was not significantly different than control mice ($2.6 \times 10^3 \pm 2.4 \times 10^2$ CFU/mL; $p = 0.91$) (Figure 3).

Residual *S. aureus* biofilm after mixture of bacteria and blood, as measured by absorbance units, was not significantly different between the CIA group (1.3 ± 0.3) and the control group (1.2 ± 0.3 ; $p=0.96$) (Figure 4).

In vivo Quantification of Bacterial Burden Using Longitudinal Bioluminescent Imaging

There was no significant difference in bioluminescent signal between the CIA group and the infected control group at all postoperative time points. Immediately following surgery (POD 0), the CIA group ($2.0 \times 10^4 \pm 1.4 \times 10^3$ photons/s/cm²/sr) showed similar bioluminescence as the

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infected control group ($1.9 \times 10^4 \pm 2.0 \times 10^3$ photons/s/cm²/sr; $p=0.92$). Peak bioluminescent signal occurred at POD 3 in both the CIA group ($4.0 \times 10^5 \pm 3.5 \times 10^4$ photons/s/cm²/sr) and the infected control group ($3.9 \times 10^5 \pm 5.6 \times 10^4$ photons/s/cm²/sr; $p = 0.93$). Immediately prior to sacrifice on POD 28, the signal in the CIA group ($7.9 \times 10^4 \pm 1.4 \times 10^4$ photons/s/cm²/sr) was similar to the infected control group ($8.0 \times 10^4 \pm 2.2 \times 10^4$; $p = 0.92$) (Figure 5A, B).

Confirmation of Bacterial Burden using Implant and Surrounding Tissue CFU Quantification

Viable *S. aureus* Xen36 CFUs were identified in 0% (0 of 2) of implants from the sterile group, 62.5% (5 of 8) of implants in the infected control group and 58.3% (7 of 12) of implants in the CIA group. No significant difference existed between the mean CFUs/mL adherent to the harvested implants in the CIA group ($8.1 \times 10^2 \pm 3.4 \times 10^2$) and the infected control group ($1.0 \times 10^3 \pm 6.0 \times 10^2$; $p = 0.82$) (Figure 6A). Similarly, no significant difference existed between the mean CFUs/mL from the harvested tissue in the CIA group ($1.1 \times 10^4 \pm 9.1 \times 10^3$) and the infected control group ($1.4 \times 10^4 \pm 9.6 \times 10^3$; $p = 0.80$) (Figure 6B).

CIA Severity Score

The mean clinical severity scores of CIA mice at each postoperative time point ranged from 5.5 to 6.25 with a range of 5 to 8 for each individual mouse (Figure 7A-C).

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Discussion

PJI is a devastating complication with poor outcomes, high patient morbidity and mortality as well as limited treatment options⁴⁸⁻⁵⁰. Given the health and economic burden of PJI to the United States healthcare system^{4-6,8}, it is of the utmost importance to thoroughly study factors that affect infectious risk. Due to articular chondrolysis, RA often results in debilitating joint pain, thus driving the need for arthroplasty in this patient population^{16,20,21}. There is currently an unmet need to thoroughly investigate the impact that RA alone, without therapeutics, has on the risk of PJI.

In this study, a well-validated animal model of human RA, along with a preclinical model of PJI was combined in a novel fashion to investigate how RA affects infectious burden after arthroplasty. CIA mice utilized in this study demonstrated paw edema scores between 5 to 8, indicating that they were clinically representative of moderately severe RA. Systemic alteration of the immunologic profile of CIA mice was confirmed as CIA mice demonstrated significantly elevated levels of systemic IgG as compared to control mice. The blood of CIA mice demonstrated similar respiratory burst capacity to the blood of control mice. Furthermore, *ex vivo* *S. aureus* growth and biofilm inhibition did not significantly differ in the CIA mice as compared to infected control mice. CIA mice also demonstrated no significantly different *S. aureus* burden *in vivo* compared to infected control mice as bioluminescent signals started, peaked and ended at remarkably similar levels at POD 0, 3 and 28, respectively. Additionally, 58.3% of implants in CIA mice showed viable, adherent *S. aureus* as compared to 62.5% of implants in the infected control group. Concurrent with these findings, implant and surrounding

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tissue *S. aureus* CFU counts 28 days after surgery and inoculation did not significantly differ between the groups. Thus, although CIA mice had an altered immune profile, no difference in *S. aureus* burden was appreciated when compared to control mice.

Taken together, the results of this study suggest the possibility that moderately severe RA alone may not represent an inherently significant infectious risk factor. As such, the perioperative management of patients with moderately severe RA may not have to be based on concern for infectious risk. When considering the perioperative infectious burden of patients with RA, it is important to consider how the upregulation of the immune response of RA weighs against its general dysregulation. If a clinical association between RA and PJI in fact exists, as has been suggested in the literature²¹⁻²⁷, it is possible that the cause of this association may be secondary to more modifiable factors, such as the perioperative use of immunosuppressive medications and secondary wound complications, rather than the disease process itself. Isolating the infectious burden of RA, alone, is the first step in developing strategies and guidelines to mitigate the risk of PJI in this population. Once this infectious risk is understood, factors such as perioperative immunomodulatory medication management as well as wound healing complications can be thoroughly investigated.

There are several limitations to this study and further translational and clinical investigation is certainly warranted. Although CIA is the gold standard animal model for studying RA, as CIA mice generate autoantibodies to collagen in a similar fashion to humans with RA, the exact immune profile of human RA remains difficult to perfectly duplicate. As such, the immune

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response in CIA mice may not perfectly replicate the immune response in human RA. Furthermore, the current study is not suited to investigate the mechanistic, molecular rationale for the findings that no increased infectious burden was seen in CIA mice. As such, this study cannot comment on how the dysregulated immune system of CIA responded to inoculation nor how it affected biofilm formation. This study also only investigated the immune response of mice that had moderately severe CIA. Although this level of severity was desired as it is moderately severe RA that causes the most uncertainty regarding optimal perioperative management, these results cannot be extrapolated to mild or severe RA. In this study, no subjective difference in wound healing was noted between experimental groups. However, wound healing in this mouse model of PJI is an imperfect representation of that which occurs following arthroplasty in humans. This is an important limitation, as patients with RA are generally reported to have higher rates of wound complications, increasing their susceptibility to PJI^{32,51,52}. Another limitation to this study is that systemic IgG levels were measured prior to surgery to confirm a molecular change in CIA mice. IgG levels were not measured after surgery and inoculation, however the aim of this study was not to analyze how these IgG levels would be affected with the introduction of bacteria. Lastly, this implant infection model is a simplification of the steps involved in joint arthroplasty, so the translational limitations of this model should be considered. The advantages of this model are widely viewed to outweigh the accepted limitations as this model allows for a safe, reproducible and well-powered manner to longitudinally quantify infectious burden *in vivo*.

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340 Despite these limitations, this study represents the first of its kind to examine the effect of RA
 341 on infectious burden in PJI using a novel combination of two well-validated animal models. The
 342 current findings provide *ex vivo* and *in vivo* evidence that mice with CIA do not demonstrate any
 343 significant difference in susceptibility to *S. Aureus* infection as compared to control mice. This
 344 suggests that moderately severe RA, alone, may not be an independent risk factor for PJI.
 345 Understanding how RA affects the risk of PJI and finding ways to mitigate this risk are crucial in
 346 developing strong strategies and guidelines for the perioperative management of this patient
 347 population.

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Figure Legends

Figure 1: ELISA demonstrating that mice with CIA had significantly higher systemic IgG levels than control mice 6 weeks after induction of CIA. ** denotes $p < 0.01$.

Figure 2: Measurement of respiratory burst *ex vivo* showing no significant difference in respiratory burst between the blood of mice with CIA when compared to control mice ($p = 0.89$).

Figure 3: Blood collection and plating procedure (A) and subsequent CFU counts demonstrating no significant difference in *S. aureus* burden *ex vivo* in the blood of mice with CIA as compared to control mice (B) ($p = 0.91$).

Figure 4: *Ex vivo* quantification of biofilm formation demonstrating no significant difference in *S. aureus* Xen36 burden between the blood of mice with CIA and control mice (A). This was measured by absorbance after staining of residual biofilm with crystal violet (B) ($p = 0.96$).

Figure 5: *S. aureus* burden *in vivo* demonstrating no significant difference between mice with CIA and infected control mice at all postoperative time points (A). Representative images depicting *in vivo* *S. aureus* Xen36 bioluminescence at three postoperative time points (B).

Figure 6: CFU quantification of *S. aureus* harvested from implants (A) and surrounding tissue (B) demonstrating no significant difference in *S. aureus* burden between mice with CIA and infected control mice ($p = 0.82$ and $p = 0.80$ for implant and tissue, respectively).

Figure 7: Mean clinical severity score of mice induced with CIA demonstrating a consistent level of moderate arthritis throughout the study period (A). Scores for each paw were given on a scale of 0-3 based on the amount of dorsal edema and added together for a total maximum score of 12. Representative images of paws of mice with CIA (B) and normal mice (C) are shown.

Quantification of Systemic IgG Levels

Figure 1: ELISA demonstrating that mice with CIA had significantly higher systemic IgG levels than control mice 6 weeks after induction of CIA. ** denotes $p<0.01$.

Ex vivo Measurement of Respiratory Burst

Figure 2: Measurement of respiratory burst ex vivo showing no significant difference in respiratory burst between the blood of mice with CIA when compared to control mice (p=0.89).

Ex vivo CFU Quantification

Figure 3: Blood collection and plating procedure (A) and subsequent CFU counts demonstrating no significant difference in *S aureus* burden ex vivo in the blood of mice with CIA as compared to control mice (B) ($p=0.91$).

Ex vivo Measurement of Biofilm Formation

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