A Novel Mouse Model of Post-Arthroplasty *Staphylococcus aureus* Infection: Real-Time *in vivo* Bioluminescence and Fluorescence Imaging To Longitudinally Track Infection

+1Berenthal, N M; 1Stavrakis, A I; 2Lieberman, J R; 2Garcia, N C; 2Kremen T J; 1Oakes, D A; 1Fineman, G A; 1Adams, J S; 1Miller, L S
+1University of California, Los Angeles, CA; 2University of Connecticut Health Center, Farmington, CT
nberenthal@mail.mednet.ucla.edu

INTRODUCTION: In the U.S., the number of joint replacements performed annually is projected to increase from 600,000 this year to more than 4 million by 2030. Post-arthroplasty infections occur in ~0.5-1% of primary joint replacements and 3-5% of revisions.1 These infections are clinically devastating, leading to extended rehabilitation, increased health care cost, and significantly worse clinical outcomes. The objectives of this study were (1) to develop a novel mouse model of post-arthroplasty *Staphylococcus aureus* infection using real-time *in vivo* bioluminescence and fluorescence imaging and (2) to assess the role of pro-inflammatory mediators in the prevention of post-surgical infection.

METHODS: All procedures were approved by the UCLA Animal Research Committee (ARC#: 2008-112). 12-week old male mice on a C57BL/6 background were used in all experiments. To simulate a post-arthroplasty infection, the distal right femur was accessed through a medial parapatellar arthrotomy with lateral displacement of the quadriceps-patellar complex. An orthopaedic-grade stainless steel Kirschner (K)-wire (diameter 0.6 mm) was placed in a retrograde fashion into the femur and cut with 1 mm protruding into the joint space (Fig 1). *S. aureus* (bioluminescent SH100 strain) (5e2 to 5e4 CFUs) in a 2 µl volume was inoculated onto the tip of the implant within the joint space. Bacterial burden within the infected post-operative knee joints was measured with *in vivo* bioluminescence imaging on post-operative days 0, 1, 3, 5, 7 and 10 (Fig 2). Neutrophil recruitment to the site of infection was simultaneously quantified by measuring *in vivo* fluorescence with the use of a mouse strain that possesses fluorescent neutrophils (lysEGFP mice). In some experiments, mice deficient in TNFα or IL-1β were used. Statistical differences between groups will be determined by multiple regression analysis, followed by a Student’s t test (two-tailed).

RESULTS: *In vivo* bioluminescence and fluorescence imaging as a measure of bacterial burden and neutrophil influx in a post-arthroplasty *S. aureus* infection model. Different logarithmic inocula of *S. aureus* (5e2 to 5e4) CFUs/2 µl of the bioluminescent *S. aureus* strain were inoculated directly onto the tip of the K-wire within the knee joint. Mice inoculated with 5e3 to 5e4 CFUs developed substantially increased bacterial burden (up to 10- to 50-fold higher than uninfected mice) (data not shown) at the site of joint infection. Clinically, these mice developed marked swelling, redness, warmth and decreased mobility of the affected leg and were euthanized on day 5 after infection. These inocula of *S. aureus* enabled measurement of the bacterial burden and neutrophil influx within the infected joints using *in vivo* imaging techniques; however, the clinical signs of infection resembled those of an acute purulent joint infection rather than those of a chronic, persistent infection.

**Chronic and persistent post-arthroplasty infection with a lower *S. aureus* inocula (5e2 CFUs) into the joint.** Mice that received 5e2 CFUs developed a detectable and measurable level of bacterial burden that peaked on day 3 and remained at an almost constant level through day 10. Clinically, these mice had some swelling and decreased mobility of the leg. However, the degree of inflammation was minimal compared with the other groups that received higher inocula. The relatively good clinical condition of these mice enabled us to follow them to day 10 when the experiment was arbitrarily terminated. Thus, an inoculum of only 500 bacteria was capable of producing a detectable and measurable post-arthroplasty bacterial infection that persisted for at least 10 days.

**DISCUSSION:**

The role of TNFα and IL-1β in host defense against post-arthroplasty *S. aureus* infection. To determine the role of pro-inflammatory mediators in protection against post-operative *S. aureus* infection in our model, TNFα-deficient, IL-1β-deficient and wildtype (wt) mice were inoculated with 5e2 CFUs of *S. aureus* after K-wire placement. IL-1β-deficient mice, but not TNFα-deficient mice, developed markedly increased bacterial signals compared with wt mice. This demonstrates an important role for IL-1β in the early control of a post-operative joint infection.

**Figure 2. In vivo bioluminescent imaging of *S. aureus* within the infected post-operative knee joints**

A. *S. aureus* bacterial counts (*in vivo* bioluminescence (max flux))

B. *S. aureus* bacterial counts (*in vivo* bioluminescence)

The role of TNFα and IL-1β in host defense against post-arthroplasty *S. aureus* infection using direct inoculation of an intraarticular K-wire and *in vivo* bioluminescence and fluorescence imaging to measure bacterial burden and neutrophil influx in real time. 5e2 CFUs of *S. aureus* produced an infection that resembled a chronic and persistent post-arthroplasty joint infection. IL-1β but not TNFα was found to be important in controlling the post-arthroplasty joint infection in our model. The advantage of this model is that it provides accurate longitudinal measurements of the joint infection burden without requiring the sacrifice of animals at different time points. This model provides a noninvasive *in vivo* system to not only investigate critical immune mechanisms, but also evaluate various antibiotic and antimicrobial coatings that may protect against post-operative bacterial infection after total joint replacement.