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Protective role of IL-1β against post-arthroplasty *Staphylococcus aureus* infection

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Abstract

MyD88 is an adapter molecule that is used by both IL-1R and TLR family members to initiate downstream signaling and promote immune responses. Given that IL-1β is induced after *S. aureus* infections and TLR2 is activated by *S. aureus* lipopeptides, we hypothesized that IL-1β and TLR2 contribute to MyD88-dependent protective immune responses against post-arthroplasty *S. aureus* infections. To test this hypothesis, we used a mouse model of a post-arthroplasty *S. aureus* infection to compare the bacterial burden, biofilm formation and neutrophil recruitment in IL-1β-deficient, TLR2-deficient and wildtype mice. By using *in vivo* bioluminescence imaging, we found that the bacterial burden in IL-1β-deficient mice was 26-fold higher at 1 day after infection and remained 3- to 10-fold greater than wildtype mice through day 42. In contrast, the bacterial burden in TLR2-deficient mice did not differ from wildtype mice. In addition, implants harvested from IL-1β-deficient mice had more biofilm formation and 14-fold higher adherent bacteria compared with those from wildtype mice. Finally, IL-1β-deficient mice had ~50% decreased neutrophil recruitment to the infected postoperative joints than wildtype mice. Taken together, these findings suggest a mechanism by which IL-1β induces neutrophil recruitment to help control the bacterial burden and the ensuing biofilm formation in a post-surgical joint.

Keywords

Staphylococcus aureus; arthroplasty; joint; TLR2; IL-1β

INTRODUCTION

Despite the widespread use of intravenous antibiotic prophylaxis and a focus on aseptic surgical technique, post-arthroplasty infections still occur in ~1.2% of primary arthroplasties and 3–5% of revisions.[1,2] The number of these infections is projected to increase to 266,000 per year by 2030 as the need for arthroplasty in the aging population will exceed 3.8 million surgeries.[3,4] The treatment of a post-arthroplasty infection is extremely difficult, as invading

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bacteria form biofilms on implanted foreign materials that block penetration of immune cells and antibiotics.[5,6] In the U.S., a two-stage surgical procedure is the standard treatment of care, which involves: (1) surgical removal of all prosthetic components with thorough debridement, placement of an antibiotic-impregnated spacer, administration of a 6-week course of intravenous antibiotics, and (2) revision arthroplasty after the infection has cleared.[5,6] Taken together, the treatment of post-arthroplasty infection involves extensive medical and surgical care, enormous health care costs, prolonged disability/rehabilitation, and significantly worse outcomes.[5,6]

Staphylococcal species, including *S. aureus* and *S. epidermidis*, account for up to 70% of post-arthroplasty infections[7,8] and an increasing proportion are due to methicillin-resistant *S. aureus* (MRSA).[9] Recent evidence has demonstrated that humans and mice deficient in the signaling adapter molecule, myeloid differentiation factor 88 (MyD88), are highly susceptible to *S. aureus* infections.[10,11] MyD88 signaling, which triggers a pathway that leads to NF-κB-mediated transcription of proinflammatory cytokines, chemokines and adhesion molecules, is activated by Toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) family members.[12,13] Relevant to *S. aureus* infections, TLR2 recognizes *S. aureus* lipopetides and lipoteichoic acid[14,15] and IL-1β is induced during *S. aureus* infections,[16,17] including *S. aureus*-infected joint tissue in patients.[18] Furthermore, IL-1β plays a protective role in mouse and rabbit models of *S. aureus* septic arthritis.[16,19] However, little is known as to whether these pathways that activate MyD88 are important for protective immunity against a post-arthroplasty *S. aureus* infection. Thus, we chose to evaluate the mechanism by which TLR2 and IL-1β play a role in host defense using an *in vivo* mouse model of post-arthroplasty *S. aureus* infection.

METHODS

Staphylococcus aureus bioluminescent strain

The bioluminescent *S. aureus* strain Xen36 (Caliper Life Sciences) was used in all experiments. This strain was derived from the parental strain ATCC 49525 (Wright), a clinical isolate obtained from a patient with *S. aureus* bacteremia. Xen36 emits a blue-green light with a peak at 490 nm because it contains the bioluminescent *luxABCDE* operon modified from *Photorhabdus luminescens* in a stable bacterial plasmid that is maintained in all progeny. This strain has been previously used to investigate *S. aureus* infections in models of bone allografts and osteomyelitis.[20,21]

Preparation of *S. aureus* for inoculation

Xen36 was streaked onto tryptic soy agar plates (tryptic soy broth [TSB] plus 1.5% bacto agar [BD Biosciences]) and grown at 37°C overnight as previously described.[22] Single colonies of Xen36 were cultured in TSB and grown overnight at 37°C in a shaking incubator (240 rpm) (MaxQ 4450; Thermo). Mid-logarithmic phase bacteria were obtained after a 2 h subculture of a 1/50 dilution of the overnight culture. Bacterial cells were pelleted, resuspended and washed 3x in PBS. Bacterial concentrations were estimated by measuring the absorbance at 600 nm (Biomate 3; Thermo). Colony forming units (CFUs) were verified after overnight culture of plates.

Mice

12 week old male congenic mice on a C57BL/6 genetic background were used in all experiments. IL-1β–deficient mice (F8) have been previously described.[17] TLR2-deficient mice (B6.129-*Tlr2*^{tm1Kir}/J) (F7) and wildtype (wt) C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME). All mouse colonies were maintained at UCLA in autoclaved cages under specific pathogen–free conditions.

Mouse surgical procedures

All procedures were approved by the UCLA Animal Research Committee. To model a post-arthroplasty *S. aureus* infection, an orthopaedic-grade stainless steel Kirscher-wire (K-wire) (0.6 mm in diameter; Synthes) was surgically placed into the right knee joint by accessing the distal right femur through a medial parapatellar arthrotomy as previously described.[22] A femoral medullary canal was manually reamed with a 25-gauge needle and the K-wire was press-fit in a retrograde fashion and cut with 1 mm protruding into the joint space. An inoculum of Xen36 (1×10^3 CFUs) in 2 μl of saline was pipetted into the joint space containing the cut end of the implant and the surgical site was closed with Vicryl 5-0 sutures. Buprenorphine (0.1 mg/kg) was administered as an analgesic subcutaneously every 12 hours for 14 days. For *in vivo* bioluminescence, the mice were followed for 42 days. To evaluate biofilm formation, implanted pins were evaluated at an early (day 7) and late (day 42) time point. CFUs of bacteria adherent to the implant were determined on day 42. To evaluate neutrophil recruitment, which occurs early on after the inoculation, the joint tissue was harvested on day 1.

In vivo bacterial burden as measured by *in vivo* bioluminescence imaging

Mice (n=8 per group) were anesthetized with inhalation isoflurane (2%) and *in vivo* bioluminescence imaging was performed using the Xenogen IVIS Lumina® imaging system (Caliper Life Sciences) as previously described.[22] Data are presented on color scale overlaid on a grayscale photograph of mice and quantified as maximum flux (photons per second (s) per cm^2 per steradian (sr) [$p/s/cm^2/sr$]) within a circular region of interest (1×10^3 pixels) using Living Image® software (Xenogen).

Variable-pressure scanning electron microscopy

Mice (n=3 per group) were euthanized on days 7 and 42, implants were harvested and biofilm formation on the intra-articular end of the implants were visualized using a field emission variable-pressure scanning electron microscope (VP-SEM) (FE-SEM Zeiss Supra VP40) as previously described.[22]

Quantification of adherent *S. aureus* bacteria on the implants

Bacteria adherent to the implants (n=5 per group) were quantified by detaching the bacteria from the implants harvested on day 42 by sonication in 1 ml 0.3% Tween-80 in TSB for 10 minutes followed by vortexing for 5 minutes and serial dilutions were plated and cultured overnight as previously described.[22]

Histologic analysis

Mice (n=3 per group) were euthanized on day 1 and infected joint tissue specimens were fixed in formalin (10%) overnight. Specimens were decalcified by incubation in Decalcifier II® solution (Surgipath) for 6 h and specimens were processed and embedded in paraffin. Sagittal sections (4 μm) were cut and stained with hematoxylin and eosin (H&E). Photomicrographs were obtained using a Leica DM2500 light microscope equipped with a DFC230 camera (Leica Microsystems).

Myeloperoxidase activity

Mice (n=5 per group) were euthanized on day 1 and infected joint tissue specimens were homogenized (Pro200® Series homogenizer; Pro Scientific). The tissue homogenate was centrifuged at 12,000 × g for 15 minutes at 4°C and supernatants were assayed for myeloperoxidase activity levels (ng/mg tissue) using the EnzChek® Myeloperoxidase Activity Assay Kit, according to the manufacturer's instructions (Invitrogen).

Statistical analysis

Data were compared by using a Student's *t*-test (two-tailed). All data are expressed as mean ± standard error of the mean (sem) where indicated. Values of $p < 0.05$ were considered statistically significant.

RESULTS

IL-1β-deficient mice had increased *in vivo* bacterial burden compared with TLR2-deficient mice or wt mice

In the presence of an orthopaedic-grade implant, intraoperative knee joints of IL-1β-deficient, TLR-deficient and wt mice (n=8 per group) were inoculated with 1×10^3 CFUs of *S. aureus* strain Xen36, which contains a stable bioluminescent construct. *In vivo* bioluminescence imaging (Xenogen IVIS; Caliper Life Sciences) was used to determine the bacterial burden *in vivo* in anesthetized mice in real-time. Using this mouse model of post-arthroplasty infection, we previously determined that *in vivo* bioluminescence signals highly correlate with the bacterial CFUs harvested from infected knee joints.[22] We found that IL-1β-deficient mice had a 26-fold greater bacterial burden compared with wt mice at day 1, which remained 3- to 10-fold greater than wt mice through day 42 ($p<0.05$) (Fig. 1). In contrast, the bacterial burden in TLR2-deficient mice did not differ from wt mice. These data demonstrate that IL-1β-deficient mice (but not TLR2-deficient mice) had higher bacterial burden than wt mice at all time points through postoperative day 42. Since the bacterial burden in TLR2-deficient mice did not differ from wt mice, the remaining experiments were designed to determine the mechanism for the higher bacterial burden observed in IL-1β-deficient mice.

IL-1β-deficient mice had substantially more biofilm formation and adherent bacteria on the implants than wt mice

On postoperative days 7 and 42, implants harvested from IL-1β-deficient mice and wt mice (n=3 per group) had detectable biofilm formation as visualized by VP-SEM. However, IL-1β-deficient mice had markedly more biofilm formation than wt mice at both time points (Fig. 2A). To determine the numbers of bacteria present in the biofilms, implants (n=5 per group) were harvested at day 42 from IL-1β-deficient mice and wt mice (Fig. 2B). IL-1β-deficient mice had 14-fold higher bacterial CFUs adherent to the implants compared with wt mice ($p<0.05$). These results demonstrate that the more pronounced biofilms in IL-1β-deficient mice observed by VP-SEM harbor increased numbers of bacteria.

IL-1β-deficient mice had decreased neutrophil recruitment to the infected knee joints compared with wt mice

A critical first-line of defense is neutrophil recruitment to the site of a *S. aureus* infection, as demonstrated by the severe joint infections in mice depleted of neutrophils.[19,23] To determine the degree of neutrophil recruitment, infected post-operative joint tissue from IL-1β-deficient and wt mice (n=3 per group) was obtained on day 1 and analyzed by histology (Fig. 3A). IL-1β-deficient mice had markedly less neutrophils within the infected joint tissue than wt mice. To quantify the number of neutrophils within the infected joints, myeloperoxidase (MPO) activity, which closely approximates neutrophil number, was determined on homogenized joint tissue from IL-1β-deficient and wt mice (n=5 per group) (Fig. 3B). IL-1β-deficient mice had ~50% less MPO activity in the infected joint tissue compared with wt mice ($p<0.001$). Thus, both histology and MPO assays demonstrated that IL-1β-deficient mice have significantly less neutrophil recruitment to the infected knee joints than wt mice.

DISCUSSION

Infection after total joint arthroplasty is a disastrous complication. Treatment is extremely challenging and time-consuming, health care costs are enormous and patient outcomes are worse.[5,6] While much time and energy has been spent on evaluating prophylactic strategies to prevent an infection,[5,6] very little is known about the role of the immune response in combating these infections. Recent evidence has demonstrated that humans and mice deficient in MyD88 are highly susceptible to *S. aureus* infections.[10,11] Therefore, we chose to evaluate the contribution of IL-1β and TLR2 in the immune response during a post-arthroplasty infection since they utilize MyD88 to initiate signaling and they have been previously implicated in host defense against *S. aureus* infections in various organs and tissues.[16–19]

Using a mouse model of post-arthroplasty *S. aureus* joint infection,[22] we were able to study the roles of these proinflammatory mediators in both the early and late stages of the immune response to *S. aureus* in a post-surgical joint in the presence of a metallic implant. We found that IL-1β-deficient mice but not TLR2-deficient mice had markedly increased bacterial burden, which was most pronounced on day 1 but persisted through day 42. Furthermore, more prominent biofilm formation and higher numbers of adherent bacteria were observed on implants harvested from IL-1β-deficient mice compared with those from wt mice. Finally, analysis of MPO activity and histologic studies showed a significant decrease in neutrophil recruitment to the infected joints of IL-1β-deficient mice compared with those of wt mice.

Taken together, these findings demonstrate an important role for IL-1β in the early control of bacterial burden in a post-surgical joint. Furthermore, these data suggest a mechanism by which IL-1β mediates its protective effect through promoting neutrophil recruitment to the site of infection. This neutrophilic response likely contributes to controlling the bacterial growth and decreasing the ensuing biofilm formation. Interestingly, mice deficient in TLR2, which promotes immune responses through the same MyD88-signaling pathway as IL-1β, did not show an increased bacterial burden compared to wildtype mice. This finding is consistent with a recent report in humans demonstrating that a TLR2 polymorphism, which rendered this receptor dysfunctional, had no impact on the risk or outcome of post-arthroplasty *S. aureus* infections.[24]

It is tempting to speculate that manipulation of the IL-1β pathway could provide a therapeutic advantage to help prevent post-arthroplasty infections. However, enhancing the inflammatory response may have unwanted consequences in arthroplasty, especially since inflammatory cells (especially macrophage-induced inflammation) promote periprosthetic osteolysis that leads to implant loosening and failure.[25] Indeed, IL-1β is upregulated in tissue surrounding failed total joint replacement implants and has been implicated in periprosthetic osteolysis.[26–28] Thus, any therapeutic strategy would need to enhance the early protective IL-1β response while minimizing any sustained inflammation that would compromise the success of the implant.

Acknowledgments

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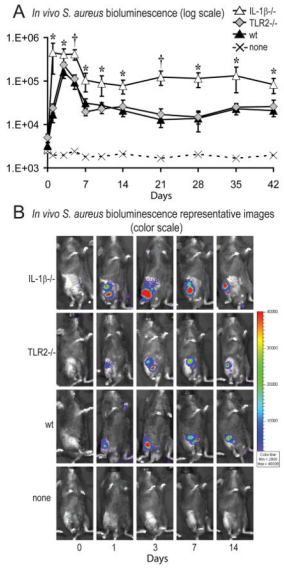

Figure 1. IL-1β-deficient mice had increased *in vivo* bacterial burden compared with TLR2-deficient mice or wt mice

The right knee joints of IL-1β$^{-/-}$, TLR2$^{-/-}$ and wt mice were inoculated with 1×10^3 CFUs of *S. aureus* (n=8 mice/group) in the presence of an orthopaedic-grade K-wire implant. (A) Bacterial counts as measured by *in vivo S. aureus* bioluminescence (mean maximum flux [p/s/cm2/sr] ± sem) (logarithmic scale). †p<0.01, *p<0.05 IL-1β$^{-/-}$ versus wt mice. (B) Representative *in vivo S. aureus* bioluminescence on a color scale overlaid on top of a grayscale image of mice.

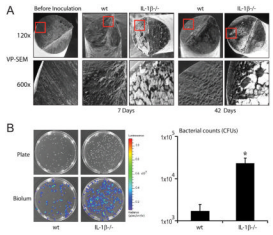

Figure 2. IL-1β-deficient mice had substantially more biofilm formation on the implants than wt mice

The right knee joints of IL-1$\beta^{-/-}$ and wt mice were inoculated with 1×10^3 CFUs of *S. aureus* (n=3 mice/group) in the presence of an orthopaedic-grade K-wire implant. (A) Representative VP-SEM images of the biofilms on the intra-articular ends of the implants harvested from infected joints on postoperative days 7 and 42 are shown (1 of 3, with similar results). Top panels represent a low magnification (120x) and the bottom panels show a higher magnification (600x) of the area boxed in red. (B) Representative plates and bioluminescent colonies (left panels) and numbers of CFUs (right panel) of bacteria released from the implants (n=5 mice/group) on day 42 after overnight culture. *$p<0.05$ IL-1$\beta^{-/-}$ versus wt mice.

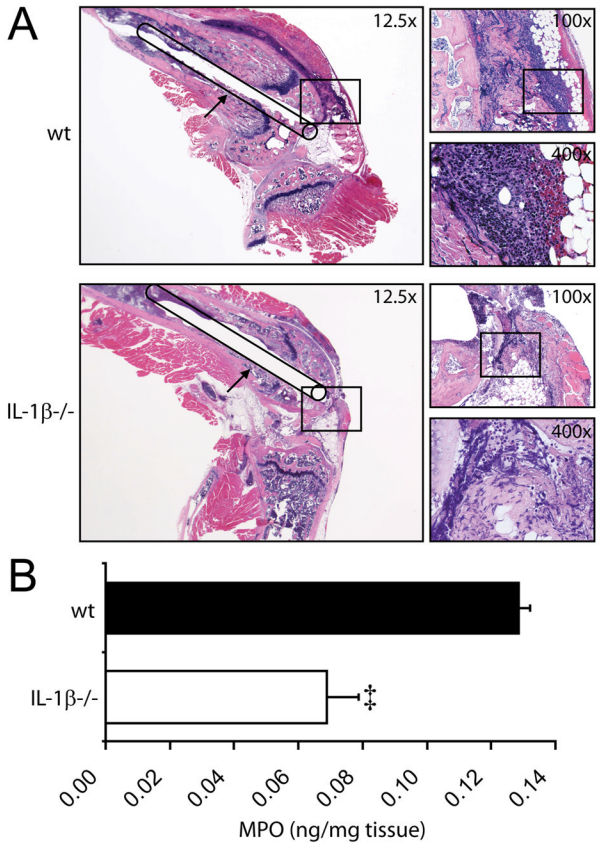

Figure 3. IL-1β-deficient mice had decreased neutrophil recruitment to the infected knee joints compared with wt mice

The right knee joints of IL-1β$^{-/-}$ and wt mice were inoculated with 1×10^3 CFUs of *S. aureus* in the presence of an orthopaedic-grade K-wire implant. The infected joint tissue was harvested on postoperative day 1. (A) Representative photomicrographs of histologic sagittal sections of wt mice (top panels) and IL-1β$^{-/-}$ mice (bottom panels) are shown (1 of 3 mice per group, with similar results). Left large panels: low magnification (12.5x) of H&E-stained joint specimens with a line drawing of the location of the implant extending into the joint from the femoral canal. Upper right small panels: higher magnification (100x) of H&E-stained joint specimens of the boxed area in the left panel at the location of the intra-articular end of the implant. Lower right small panels: higher magnification (400x) of H&E-stained sections in the boxed areas in the upper right panels. (B) Mean myeloperoxidase activity of the infected joint tissue specimens (ng/mg of tissue) ± sem (n=5 per group). ‡$p<0.001$ IL-1β$^{-/-}$ versus wt mice.