Systemic IL-27 administration prevents abscess formation and osteolysis via local neutrophil recruitment and activation

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Interleukin-27 is a pleiotropic cytokine whose functions during bacterial infections remain controversial, and its role in patients with S. aureus osteomyelitis is unknown. To address this knowledge gap, we completed a clinical study and observed elevated serum IL-27 levels (20-fold higher, P < 0.05) in patients compared with healthy controls. Remarkably, IL-27 serum levels were 60-fold higher in patients immediately following septic death than in uninfected patients (P < 0.05), suggesting a pathogenic role of IL-27. To test this hypothesis, we evaluated S. aureus osteomyelitis in WT and IL-27Ra−/− mice with and without exogenous IL-27 induction by intramuscular injection of rAAV-IL-27p28 or rAAV-GFP, respectively. We found that IL-27 was induced at the surgical site within 1 day of S. aureus infection of bone and was expressed by M0, M1 and M2 macrophages and osteoblasts but not by osteoclasts. Unexpectedly, exogenous IL-27p28 (~2 ng·mL−1) in serum delivery ameliorated soft tissue abscesses and peri-implant bone loss during infection, accompanied by enhanced local IL-27 expression, significant accumulation of RORγt+ neutrophils at the infection site, a decrease in RANK+ cells, and compromised osteoclast formation. These effects were not observed in IL-27Ra−/− mice compared with WT mice, suggesting that IL-27 is dispensable for immunity but mediates redundant immune and bone cell functions during infection. In vitro studies and bulk RNA-seq of infected tibiae showed that IL-27 increased nos1, nos2, il17a, il17f, and rorc expression but did not directly stimulate chemotaxis. Collectively, these results identify a novel phenomenon of IL-27 expression by osteoblasts immediately following S. aureus infection of bone and suggest a protective role of systemic IL-27 in osteomyelitis.

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INTRODUCTION

Despite significant medical advances, deep bone infections continue to be the bane of orthopedic surgery, with infection rates essentially remaining at 1%–2% after elective surgery over the past 50 years1–4. Staphylococcus aureus is the major pathogen in orthopedic infections. It is responsible for 10 000–20 000 prosthetic joint infections (PJIs) annually in the United States5,6 and 30%–42% of fracture-related infections (FRIs)7,8. Unfortunately, these difficult-to-treat S. aureus bone infections are associated with poor clinical outcomes and high recurrence rates following revision surgery9,10. With the increasing incidence of methicillin-resistant S. aureus (MRSA) osteomyelitis and emerging strains with pan-drug resistance11,12, there is an urgent need for novel immunotherapies to supplement existing antibiotic therapies.

S. aureus causes the most lethal form of human sepsis, with a 10% mortality rate, and a catastrophic outcome of osteomyelitis is death due to sepsis and multiple organ failure13,14. The cellular and molecular mechanisms underlying S. aureus osteomyelitis-induced sepsis are largely unknown. Interestingly, several studies have reported elevated serum IL-27 levels during sepsis, suggesting that IL-27 could potentially be useful in predicting sepsis-driven mortality15–20. IL-27 is a heterodimeric cytokine belonging to the IL-12 cytokine family and is mainly produced by antigen-presenting cells such as macrophages, monocytes, and dendritic cells21,22. IL-27 is composed of the IL-27p28 and EB3 subunits and signals through a heterodimeric cell surface receptor composed of IL-27 receptor α (IL-27Ra) and gp13021,22. Similar to IL-12, IL-27 signaling is mediated mainly through the intracellular JAK-STAT pathway and participates in multiple immunoregulatory activities21–23. Classical IL-27 signaling down-regulates Th17 differentiation, stimulates regulatory T-cell development, and drives IL-10 production by CD4+ T cells24. Studies involving cecal ligation and puncture (CLP)-induced bacterial sepsis and S. aureus pneumonia following influenza demonstrated that IL-27 mediates enhanced susceptibility to infection by attenuating Th17 immunity and promoting IL-10 induction26,27. These studies highlight the importance of IL-27 in immune suppression. On the other hand, IL-27 has been reported to promote the proliferation and differentiation of hematopoietic stem cells28, increase the production of proinflammatory cytokines by monocytes29,30, and induce Th1 differentiation31. Currently, the contribution of IL-27 to host immunity during S. aureus...
Effects of exogenous IL-27 in *S. aureus* osteomyelitis

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osteomyelitis is unknown. Here, we examined whether IL-27 is an essential cytokine involved in the pathogenesis of *S. aureus* osteomyelitis. We report that IL-27 expression was induced in patients with *S. aureus* osteomyelitis and that elevated serum IL-27 correlated with septic death in these patients. IL-27 is produced early in murine *S. aureus* bone infection, especially by macrophages and, surprisingly, by osteoblasts. We also unexpectedly discovered that exogenous and prophylactic administration of IL-27 significantly accelerates *S. aureus* clearance in mouse bone by enhancing early host innate immune responses and preventing bone erosion.

RESULTS

Serum IL-27 levels were associated with *S. aureus* osteomyelitis in patients

To better understand the involvement of IL-27 in host immune responses to *S. aureus* osteomyelitis, we measured IL-27 in serum from healthy individuals, orthopedic patients with culture-confirmed *S. aureus* bone infections, and patients who died from sepsis associated with *S. aureus* osteomyelitis. Serum IL-27 levels were significantly elevated in infected patients compared to uninfected individuals (20-fold higher, *P* < 0.05). Remarkably, IL-27 levels immediately following septic death were 60-fold higher than those in healthy individuals (Fig. 1a, *P* < 0.05), suggesting that IL-27 could be useful in predicting *S. aureus* osteomyelitis-induced septic death. Indeed, in this small patient cohort, formal analyses of IL-27 as a diagnostic biomarker using receiver operator characteristic (ROC) curve analysis revealed good prediction accuracy for identifying *S. aureus* osteomyelitis, as indicated by the area under the curve (AUC) of 0.922 (Fig. 1b, *P* < 0.000 1).

IL-27R is required for *S. aureus*-driven early IL-27 expression in mice

Given the association between IL-27 expression and *S. aureus* osteomyelitis in patients, we next measured IL-27 in mice with *S. aureus* osteomyelitis induced using a well-established transistibial model. C57BL/6 (WT) and *IL-27Ra*−/− mice were challenged with bioluminescent MRSA (USA300 LAC::lux), and tibiae were harvested 14 days post-infection to assess the bacterial load and measure IL-27 levels via ELISA. We observed early induction of IL-27 expression in tibiae due to *S. aureus* infection, and this response was autoregulated by IL-27/IL-27R signaling, as IL-27 induction on Day 1 was not observed in *IL-27Ra*−/− mice (Fig. 2a, *P* < 0.05). The ex vivo effects of exogenous IL-27 in the implants, tibiae, and soft tissues were not different in *IL-27Ra*−/− mice compared to WT animals (Fig. S1), suggesting that IL-27 is dispensable for clearance of *S. aureus* from bone.

*S. aureus* induces IL-27 secretion in macrophages and osteoblasts but not in osteoclasts

Although early induction of IL-27 by *S. aureus* infection of bone was evident, its cellular origin was unclear. Thus, to assess the potential of different cell populations to produce IL-27 in response to *S. aureus*, we measured IL-27 production by murine osteoblasts, osteoclasts, and macrophages in vitro. *S. aureus* induced significant concentration-dependent IL-27 secretion 24 h post-infection in both RAW 264.7 macrophages and M0, M1, and M2 murine macrophages (Fig. 2b, *P* < 0.05). Surprisingly, murine calvarial MC3T3-E1 osteoblasts and primary bone marrow-derived osteoblasts produced significantly higher amounts of IL-27 at 24 h post-infection (Fig. 2c, *P* < 0.05). In sharp contrast, bone marrow-derived osteoclasts did not produce IL-27 when exposed to *S. aureus* in vitro (Fig. 2d, *P* < 0.05). To our knowledge, this is the first demonstration of IL-27 expression by osteoblasts and suggests an important role in osteoblasts against *S. aureus* infection.

Systemic IL-27 inhibits draining abscess formation and bone loss during the establishment of *S. aureus* osteomyelitis

We next examined whether systemic IL-27 mediates bacterial clearance during *S. aureus* osteomyelitis in our murine model. MRSA (USA300 LAC::lux) was used to induce transistibial osteomyelitis in mice after intramuscular (IM) injection of recombinant adeno-associated virus expressing GFP (rAAV-GFP, control) or rAAV-IL-27p28 (Fig. 3a). We first confirmed the magnitude and stability of IL-27 production after rAAV-IL-27p28 injection and detected a temporal increase in serum IL-27, which peaked (~2 ng·mL−1) on Day 24 post-injection (Fig. 3b). While rAAV-IL-27p28 treatment did not show an effect on in vivo *S. aureus* growth, as assessed by the bioluminescence intensity (BLI) values (Fig. 3c), rAAV-IL-27p28-treated mice showed improved body weight recovery following septic surgery compared to rAAV-GFP-treated animals (Fig. 3d, *P* < 0.05). Remarkably, mice injected with rAAV-IL-27 showed much smaller draining abscesses at the sites of bone infection (Fig. 3e). Ex vivo CFU analysis confirmed that the bacterial load in surgical site soft tissues was significantly lower in rAAV-IL-27p28-treated mice (Fig. 3f, *P* < 0.05). Moreover, high-resolution μCT demonstrated that peri-implant osteolysis was
decreased in mice treated with rAAV-IL-27p28 compared to mice receiving rAAV-GFP (Fig. 3g).

MRSA-infected tibiae from rAAV-IL-27p28- and rAAV-GFP-treated mice were harvested on Days 1, 3, 7, and 14 post-septic surgery and subjected to bulk RNA sequencing. As expected, IL-27 expression in the infected tibiae was significantly upregulated in mice receiving rAAV-IL-27p28 compared to mice receiving rAAV-GFP at all time points (Fig. 3h), suggesting a positive feedback effect\(^37\). To validate the transcriptome data, the IL-27p28 protein level was measured at early time points in mouse tibia homogenates using ELISA (Fig. S2). Collectively, these results demonstrated that IL-27 affects abscess formation and bone osteolysis. Of note, CFU quantification in the implants revealed similar bacterial loads between the groups, suggesting that systemic IL-27 treatment does not affect biofilm formation on implants. Indeed, scanning electron microscopy (SEM) confirmed these findings (Fig. S3).

IL-27 enhances the accumulation of ROR\(\gamma\)\(^+\) neutrophils at early stages of implant-associated osteomyelitis

We next explored possible scenarios that can lead to the observed suppression of \(S.\) aureus Staphylococcus abscess...
Fig. 3  Systemic IL-27p28 induces local IL-27 expression and ameliorates soft tissue infection at the surgical site and osteolysis during S. aureus implant-associated osteomyelitis. a Schematic illustration of the experimental design in which 8-week-old female C57BL/6 WT and IL-27Rα−/− mice (C57BL/6J background) received an injection of rAAV-IL-27p28 or rAAV-GFP in the quadriceps 1 week prior to challenge with a MRSA (5 × 10^5 CFU of USA300 LAC::lux)-contaminated transtibial implant. The time points for the various outcome measures are also indicated. b WT mice were infected with 0.5 × 10^12 genome copies/mouse rAAV-IL-27p28 (n = 3) or rAAV-GFP (control, n = 5) via intramuscular injection, and serum samples were collected longitudinally to assess IL-27 levels via ELISA. The exogenous IL-27p28 levels in serum are presented as the means ± SDs. c–f A separate cohort of mice were infected with rAAV-IL-27p28 or rAAV-GFP via injection and subjected to transtibial implant challenge (n = 16). The longitudinal BLI (c) and total body weight (d) values are presented as the means ± SDs (*P < 0.05 on Day 3, ***P < 0.001 on Day 10 and 14, two-way ANOVA). e Photographs of representative infected tibiae obtained on Day 14 are presented to show the large vs. small draining abscesses observed in rAAV-GFP- vs. rAAV-IL-27p28-treated mice. The mice were euthanized on Day 14, and the infected tibiae were harvested for CFU (f) and micro-CT (g) analyses. CFUs in the implant, soft tissue and tibia were quantified, and the data for each tibia are presented with the mean ± SD for the group (n = 10, ****P < 0.0001, t-test). Representative 3D renderings of extensive peri-implant osteolysis and reactive bone formation in rAAV-GFP- vs. rAAV-IL-27p28-treated tibiae are shown with volumetric bone loss in infected tibiae. Data are presented for each tibia with the mean ± SD for the group (n = 6, *P < 0.05, t-test). h Total RNA was extracted from infected tibiae (n = 3) at different time points, and IL-27 mRNA levels were measured by bulk RNA sequencing. The expression data are presented as the normalized read counts with the mean ± SD for the group (**P < 0.0001 on Days 1 and 3, ***P < 0.001 on Days 7 and 14).
communities (SACs) and reduced bone loss at the surgical site. Immunohistopathology of infected tibiae revealed an increased number of neutrophils (Ly6G^+ cells) on Day 1 post-surgery in both SACs and the adjacent bone marrow in rAAV-IL-27p28-treated animals compared to rAAV-GFP control animals (Fig. 4a). Histomorphometric quantification confirmed the significant increase in neutrophils in SACs and in the bone marrow in the rAAV-IL-27 group (Fig. 4b, P < 0.05). Interestingly, increased numbers of Ly6G^+ RORγ^+ neutrophils were observed in the rAAV-IL-27p28 group (Fig. 4a, b, P < 0.05), suggesting a possible involvement of proinflammatory IL-17 signaling in bacterial clearance. Indeed, transcriptome analyses revealed increased expression of proinflammatory *IL17A*, *IL17F*, and *RORC* (which encodes the IL-17 transcription factor RORγ^38^) genes in rAAV-IL-27p28-treated mice early during infection (Fig. 4c). As expected, these genes were downregulated at the later stages of infection due to reduced bone disease. Similar trends were observed for immunostimulatory genes associated with Toll-like receptor (TLR) and iNOS signaling, suggesting an IL-27-mediated proinflammatory innate response (Fig. 4c). Indeed, we confirmed that stimulation with a combination of IL-27 and the TLR agonist lipopolysaccharide (LPS) increased nitric oxide (NO) production.
in primary macrophages, revealing the synergistic properties of IL-27 in enhancing TLR-driven production of microbicidal nitric oxide (Fig. S4).

Systemic IL-27 reduces bone loss and osteoclast formation during implant-associated osteomyelitis. μCT demonstrated that peri-implant osteolysis was ameliorated in infected mice subjected to rAAV-IL-27p28 treatment (Fig. 3g). We hypothesized that the decreased bone loss was due to reduced osteoclast formation and differentiation. Histomorphometric analysis of tibial sections stained for tartrate-resistant acid phosphatase (TRAP) and immunostained for RANK confirmed decreased osteoclast formation and resorption of trabecular bone due to systemic IL-27 treatment (Fig. 5b, c).

The effects of IL-27 on S. aureus osteomyelitis are dependent on the IL-27/IL-27R axis. It is plausible that IL-27 could directly be involved in the recruitment of neutrophils to sites of S. aureus infection. Thus, we examined whether IL-27 is chemotactic for neutrophils. An in vitro chemotaxis assay using granulocytic HL-60 cells revealed that IL-27 did not promote the migration of granulocytes through the Boyden chamber membrane (Fig. S5). IL-27 was also not chemotactic for primary bone marrow-derived macrophages (data not shown). Alternatively, it is possible that IL-27/IL-27R signaling could extrinsically induce chemotaxis of innate immune cells to the infection site. Therefore, we repeated the in vivo S. aureus osteomyelitis experiments using IL-27 receptor α knockout (IL-27Rα−/−) mice. Fourteen days post-infection, the body weight changes (Fig. 6a) and BLI values (Fig. 6b) were similar between IL-27Rα−/− mice treated with rAAV-IL-27p28 and those treated with rAAV-GFP. Most interestingly, the ex vivo CFUs in the implants, surgical site soft tissues, and tibiae were similar in IL-27Rα−/− mice (Fig. 6d). Furthermore, no differences were detected between the groups in the formation of draining abscesses on these implants (Fig. 6c) or in peri-implant osteolysis (Fig. 6e). These data indicate that the effects of rAAV-IL-27p28 on S. aureus osteomyelitis in WT mice are mediated by IL-27/IL-27R signaling.

**DISCUSSION**

Cytokines, including IL-27, are central for the timely induction of immune responses during infection. Thus, elucidation of the functions of IL-27 in the context of infection is essential to improve our understanding of protective vs. pathogenic host immunity. In this study, we measured systemic levels of IL-27 in serum from patients with S. aureus osteomyelitis. These clinical studies revealed elevated serum IL-27 levels in patients with S. aureus bone infections. In mice, we demonstrated that IL-27 expression is induced early during S. aureus infection in macrophages and osteoblasts. Remarkably, exogenous IL-27 treatment decreased the severity of S. aureus osteomyelitis, including reductions in abscess formation and bone loss. The observed phenotype was likely linked to an IL-17-mediated proinflammatory neutrophil response.

A notable finding of our study is that serum IL-27 levels were highly associated with S. aureus osteomyelitis in patients (AUC = 0.922). Previous studies have shown that serum IL-27 levels are elevated in sepsis patients, indicating the potential of this cytokine as a diagnostic biomarker for sepsis. A single-center prospective study demonstrated that serum IL-27 levels had an AUC of 0.75 in patients with sepsis. Although IL-27 levels were 60-fold higher in patients immediately following septic death than in uninfected patients, we could not calculate AUC values due to the low number of patients with septic death. However, our study indicates that IL-27 could be an diagnostic marker associated with S. aureus osteomyelitis and could help to predict septic complications. However, extensive patient cohort studies are required to formally assess its diagnostic potential.

S. aureus can persist intracellularly in osteoblasts, contributing to chronic osteomyelitis. This persistence can lead to the induction of osteoclastogenic and inflammatory cytokine expression, osteoblast apoptosis, and increased antibiotic tolerance. A remarkable finding of our study is that S. aureus induces significant levels of proinflammatory IL-27 expression in osteoblasts but not in osteoclasts. Osteoclasts, although capable of intracellular S. aureus uptake, exhibit diminished bactericidal activity compared to that of bone marrow-derived innate immune cells. Moreover, we observed that exogenous expression of IL-27 contributes to increased neutrophil accumulation and impaired osteoclast formation in the bone marrow milieu. Perhaps IL-27 treatment could promote active S. aureus uptake by osteoblasts and bone marrow innate immune cells rather than by osteoclasts. However, our results indicate an important role for this cytokine in orchestrating bone homeostasis and remodeling during osteomyelitis.

S. aureus-infected tibiae treated with rAAV-IL-27p28 exhibited substantial accumulation of neutrophils expressing RORyt, which are an innate source of IL-17, and induction of IL-17, TLR, iNOS signaling genes early during infection. These results indicate an IL-27-mediated early innate immune response driving bacterial clearance in these animals. However, transcriptomic analyses also revealed suppression of these genes at later time points during the chronic phase. From these observations, it is conceivable that IL-27 exhibits time-dependent functions in host immunity, ranging from protective immunity in acute S. aureus osteomyelitis to suppressive immunity during chronic infection. A recent study using a murine model of intrafemoral osteomyelitis demonstrated similar time-dependent changes in the host response during S. aureus osteomyelitis using gene expression analyses.

IL-27 is known to directly inhibit the early stages of RANKL-induced osteoclastogenesis and suppress osteoclast formation. Here, we observed that systemic rAAV-IL-27p28 treatment led to impaired osteoclast formation and differentiation, suppressed RANK signaling, and reduced bone osteolysis in S. aureus-infected mice. IL-27 expression studies in other disease models have shown similar marked reductions in bone loss and in peri-implant osteolysis. In murine models of collagen-induced arthritis, researchers observed that IL-27 was expressed in rheumatoid arthritis synovial membranes and that ectopic IL-27 expression decreased disease severity compared to that in untreated control mice.

In our study, it is conceivable that the observed reduction in bone loss in rAAV-IL-27p28-treated mice was due to reduced infection and not due to the direct suppressive effects of IL-27 on osteoclast formation. Additional studies at the cellular level are required to confirm the effects of IL-27 on osteoclastogenesis during S. aureus osteomyelitis.

Systemic IL-27 delivery led to amelioration of soft tissue infection at the surgical site and peri-implant bone loss in animals with S. aureus osteomyelitis. However, the bacterial loads in the implant and bone were not affected by IL-27 delivery, underscoring the ability of S. aureus to invade deep within the immune-privileged environment of bone. Interestingly, the effects of IL-27 on abscess formation and bone osteolysis were lost in IL-27 receptor α knockout mice, suggesting a direct role of IL-27/IL-27R signaling in modulating immune and bone cell functions. Similarly, Wang et al. showed that administration of recombinant IL-27 improved bacterial clearance and host survival in a rodent model of Clostridium difficile infection.

In contrast, other studies reported that IL-27 blockade increased the severity of sepsis-induced myocardial dysfunction in an endotoxic shock syndrome murine model. Collectively, these studies highlight the diverse effects of IL-27 in various bacterial infections.

To summarize our findings, we propose a schematic model of IL-27-mediated immune regulation during S. aureus osteomyelitis.

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At the onset of S. aureus osteomyelitis, osteoblasts and macrophages induce IL-27 secretion via TLR activation, and this process is dependent on the autoregulatory IL-27/IL-27Rα signaling pathway. However, endogenous IL-27 is not sufficient to influence host susceptibility to osteomyelitis. In sharp contrast, exogenous expression of IL-27 induces the accumulation of proinflammatory IL-17-producing RORγt+ neutrophils, which leads to decreased abscess formation and increased bacterial clearance at the infection site.

Fig. 5  Systemic IL-27p28 impairs osteoclast formation during implant-associated osteomyelitis. a Tibiae from the mice described in Fig. 3 were processed for histological staining for tartrate-resistance acid phosphatase (TRAP). Representative images at 2× magnification are shown (scale bars = 500 µm). b The TRAP-stained areas within the cortical bone regions, trabecular bone regions, and implant sites (red box) were quantified as percentages, and the data are presented for each tibia with the mean ± SD for the group (n = 6, **P < 0.01, t-test). c Tibia sections were also processed for immunofluorescence histochemistry for RANK, and representative images at 200× magnification are shown (scale bar = 500 µm). The white asterisk indicates the hole where the L-shaped pin was inserted. d The RANK-stained area in the bone marrow surrounding the infection site was quantified as a percentage, and the data are presented for each tibia with the mean ± SD for the group (n = 6, **P < 0.01, t-test)
Furthermore, the abundance of IL-27 suppresses RANK expression and inhibits osteoclast differentiation, leading to decreased bone osteolysis during chronic osteomyelitis. The proposed model of IL-27-mediated immune homeostasis is preliminary and warrants several future investigations. First, we utilized an AAV-IL-27p28 monomer in our studies, which could exert effects on IL-27R signaling that differ from those of the IL-27 heterodimer (p28\(^+\)EBI3\(^-\))\(^{21,65-68}\). Understanding the potential differential effects of the monomer and heterodimer in the context of \(S.\ aureus\) osteomyelitis is an important future consideration.

Second, we need to examine the complex temporal changes in the infiltrating immune cells in the bone marrow niche, which contribute to IL-27/IL-27R crosstalk during \(S.\ aureus\) osteomyelitis. Third, we need to examine specific chemotactic mechanisms that lead to the infiltration of ROYt\(^+\) neutrophils into the infection site. Finally, we need to understand how IL-27 prevents cytokine storm and internal organ tissue damage during chronic \(S.\ aureus\) osteomyelitis in a more relevant murine model of osteomyelitis-induced sepsis. Humanized mice, which are more susceptible to osteomyelitis-induced sepsis caused by MRSA, may be better suited for these studies\(^{69}\). These studies will further our understanding of IL-27/IL-27R signaling during \(S.\ aureus\) osteomyelitis.

**MATERIALS AND METHODS**

**Bacterial strains**

Methicillin-resistant \(S.\ aureus\) (USA300 LAC) was used for all in vitro experiments, and a bioluminescent strain of USA300 (USA300 LAC::lux) was used for all in vivo experiments, as previously described\(^{32-34,69}\).

**Ethics statement and patient enrollment**

Serum samples were collected from \(S.\ aureus\) osteomyelitis patients \((n = 23)\) and uninfected patients undergoing elective total joint replacement \((n = 10)\). Additionally, serum samples were collected immediately postmortem in patients who succumbed to \(S.\ aureus\) osteomyelitis sepsis \((n = 5)\). All recruited patients were either enrolled in an international biospecimen registry (AO Trauma Clinical Priority Program (CPP) Bone Infection Registry)\(^{70}\) or participated in IRB-approved clinical studies conducted at...
glutamine, 1% antimicrobial/antimycotic, and collagenase I.

The migration of osteoblasts from the bone pieces was confirmed after three to five passages were used for the IL-27 induction assays. Murine calvarial MC3T3-E1 osteoblasts were plated in DMEM + 10% FBS, 2 mmol·L\(^{-1}\) L-glutamine, and 1% antimicrobial/antimycotic to 80% confluence. To differentiate osteoclasts, bone marrow hematopoietic cells were cultured in medium containing macrophage colony-stimulating factor (M-CSF; 30 ng·mL\(^{-1}\), PeproTech), and RANKL (100 ng·mL\(^{-1}\), PeproTech). RAW 264.7 cells were cultured in DMEM + 10% FBS, 2 mmol·L\(^{-1}\) L-glutamine, and 1% antimicrobial/antimycotic to 80% confluence. Subsequently, BMDMs and RAW 264.7 cells were cultured in R10 medium containing PBS, murine IFN-γ (50 ng·mL\(^{-1}\), PeproTech) or murine IL-4 (20 ng·mL\(^{-1}\), PeproTech) for 24 h to generate M0, M1, and M2 macrophages, respectively. These cells were then infected with S. aureus USA300 at an MOI of 10 for 24 h. Following infection, cell culture supernatants were harvested for measurement of IL-27 secretion using a Mouse IL-27p28 Uncoated ELISA Kit (Invitrogen).

Serum IL-27 concentrations in patients were determined with a Luminex-based Milliplex xMAP Multiplex Assay (Millipore Sigma) according to the manufacturer’s instructions.

Nitrite production by murine macrophages Murine BMDMs were pretreated with PBS or murine IL-27 (50 ng·mL\(^{-1}\), Biologend) for 24 h and were then stimulated with or without LPS (100 ng·mL\(^{-1}\), Millipore Sigma) to induce reactive nitrogen species production\(^{19}\), which is important for the host defense against bacterial infection\(^{22}\). Additional experiments were performed utilizing BMDMs stimulated with or without murine IL-27 (50 ng·mL\(^{-1}\)) for 24 h after pretreatment. Subsequently, nitrite concentrations in the cell culture supernatant were determined with a Griess assay kit (R&D Systems).

Transwell chemotaxis assay HL-60 cells (ATCC) were differentiated into granulocytes using 100 mmol·L\(^{-1}\) dimethylformamide (DMF) (Millipore Sigma) and plated in the top compartment of Boyden chambers, and a chemotaxis assay was performed according to the manufacturer’s protocol (Millipore Sigma QCM\(^{TM}\) Chemotaxis 5 μm 24-Well Cell Migration Assay Kit). Briefly, the chemotaxis of 1 × 10^6 cells per chamber toward the bottom compartment containing

Fig. 7 Schematic model of IL-27-mediated immune regulation during S. aureus osteomyelitis. a S. aureus induces IL-27 expression via TLR activation in osteoblasts and macrophages early during the onset of osteomyelitis. Secretion of IL-27 is dependent on the autoregulatory IL-17-producing RORγt+ neutrophils, leading to decreased abscess formation and increased bacterial clearance at the site of infection. Furthermore, the abundance of IL-27 suppresses RANK expression and inhibits osteoclast differentiation, leading to decreased bone osteolysis during chronic osteomyelitis.
Intramuscular administration of the IL-27-expressing adenovirus vector (rAAV-IL-27)

To achieve sustained exogenous IL-27 expression, mice were injected intramuscularly with recombinant murine IL-27-containing AAV (IL-27 p28, 0.5 × 10^{12} genome copies per mouse, Vector Biolabs) seven days prior to surgical experiments in mice. Osteomyelitis model was utilized for all in vivo studies. C57BL/6 mice were purchased from The Jackson Laboratory and maintained in the University of Rochester animal facilities. Our well-established transplanted implant-associated osteomyelitis model was utilized for all in vivo S. aureus challenge experiments in mice. Briefer, L-shaped stainless-steel implants were contaminated with USA300 LAC:lux (5.0 × 10^5 CFU per ml) grown overnight and surgically implanted into the tibiae of 8-week-old female C57BL/6 mice from the medial to the lateral side. The body weight change and bioluminescence intensity at the infection site were evaluated longitudinally, and terminal assessment of CFUs (in the implant, surgical site soft tissue and tibia), peri-implant osteolysis (high-resolution μCT imaging), biofilm formation on the implant (Zeiss Auroga SEM imaging), and histopathology were performed on Day 14 post-septic surgery, as described previously. Murine infection studies were performed three independent times, and the data from these experiments were pooled.

Histology

Histopathological analyses were performed according to protocols described previously. Briefly, after μCT, each mouse tibia sample was fixed with 10% formalin neutral buffer for 3 days at room temperature (RT) and then decalcified with 14% ethylenediaminetetraacetic acid disodium salt dihydrate (pH 7.4) for 2 weeks at RT. All samples were embedded in paraffin and sectioned at a thickness of 5 μm. Digital images of the serially stained slides were acquired using a VS120 Virtual Slide Microscope (Olympus, Waltham, MA, USA). To compare the numbers of osteoclasts within infected tibiae, tartrate-resistant acid phosphatase (TRAP) staining was performed. The tissue sections were immersed in Antigen Unmasking Solution (Vector Laboratories) for 2 h. Then, 5% hydrogen peroxide was added to the sections for 15 min. After washing with PBS and incubation with a secondary antibody at RT for 1 h, the slides were rinsed for 1 h in PBS and mounted with Vectashield antifade mounting medium with DAPI (H-1200, Thermo Fisher Scientific). The region of interest was manually selected using a custom Analysis Program in Subread. The remaining high-quality processed reads were then mapped to the Mus musculus reference genome (GRCm38.p6) with STAR version 2.7.0f using the following parameters: two-passMode Basic --runMode alignReads --genomeDir /GENOME --readFilesIn SAM files

Statistics

For statistical analyses involving more than two groups, we utilized the nonparametric Kruskal–Wallis test, one-way ANOVA and two-way repeated measures ANOVA. Unpaired Student’s t-test was used to compare the means of two groups. Differences were considered significant at P < 0.05.
t-test was used to assess the significance of differences between two experimental groups. The data are presented as the means ± standard deviations. A P-value of <0.05 was considered significant.

DATA AVAILABLE
All RNA sequence data have been submitted to the Gene Expression Omnibus under accession number GSE168896.

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ADDITIONAL INFORMATION
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