Published in final edited form as:
Cell Host Microbe. 2013 June 12; 13(6): 629–631. doi:10.1016/j.chom.2013.05.015.

Staphylococcus aureus Osteomyelitis: Bad to the Bone

Michael E. Olson[1] and **Alexander R. Horswill**[1,*]

[1]Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA 52242, USA

Previews

Osteomyelitis is a debilitating bone infection often caused by the bacterial pathogen *Staphylococcus aureus*. In this issue, Cassat and colleagues (2013) develop a high-resolution micro-computed tomography (microCT) method to visualize bone remodeling during *S. aureus* infection and discover that the metalloprotease aureolysin plays a critical role in modulating osteomyelitis pathogenesis.

Osteomyelitis is a bacterial infection of the bone or bone marrow for an alarming number of patients that are admitted to hospitals (Lew and Waldvogel, 2004). *Staphylococcus aureus* is a prominent bacterial pathogen and the most frequent isolated causal agent of infection-induced osteomyelitis (Hatzenbuehler and Pulling, 2011). Treatment of osteomyelitis infections is challenging due a variety of factors, including the poor bioavailability of antibiotics in bone tissue, rising antibiotic resistance in bacterial pathogens, and the biofilm-like properties of the infection. In many cases, physicians are left with surgical debridement as the only option to remove the invading bacteria and achieve a sterile site. Where an infected prosthetic joint is involved, removal and replacement of the joint is often required (Zimmerli et al., 2004). Due to these challenges, osteomyelitis is devastating for many patients with painful long-term consequences and limited treatment options.

Improving the clinical outcomes of osteomyelitis infections would be a significant medical advance. Our current limited knowledge of disease progression and the availability of technologies to monitor these events is an unmet need that has hampered ongoing studies. In this issue of *Cell Host & Microbe*, Cassat *et al.* (2013) address these challenges by developing and utilizing a novel three-dimensional imaging modality, called high-resolution micro-computed tomography (microCT), that can visualize and quantify infectious damage to the bone. To perform microCT, a new model of murine osteomyelitis was developed and confirmed using a community-acquired methicillin-resistant *S. aureus* isolate of the USA300 type, an emerging cause of musculoskeletal infections (Kourbatova et al., 2005). In the murine model, a sterile 1 mm unicortical bone defect is created, serving as an *S. aureus* inoculation point to establish osteomyelitis. To monitor disease progression, microCT is performed to visualize cortical bone destruction and new bone formation, and computational algorithms translate these changes into a three-dimensional image. The end result is a visual reconstruction that captures a striking snapshot of bone remodeling as a result of *S. aureus* infection (see Figure 1). The developed imaging software provides informative volume outputs of bone loss and new bone growth that serve an important role in quantifying

*Correspondence: alex-horswill@uiowa.edu.

osteomyelitis-related damage. To verify the microCT findings, the authors (Cassat et al., 2013) performed histopathology on the murine model. Thus, the methods developed in this paper make it possible for the first time to monitor and quantify osteomyelitis disease using a powerful new imaging platform.

To gain insight on *S. aureus* mechanisms of osteomyelitis pathogenesis, Cassat *et al.* (2013) employed the microCT imaging technology on USA300 and isogenic mutants in previously described key pathogenesis regulators. Due to the limited knowledge of contributing factors to this disease, a *S. aureus* strain lacking two global virulence regulators (Agr and Sae systems) was tested in the murine model. The double mutant showed significantly less cortical bone destruction and reduced new bone formation, indicating that secreted virulence factors were likely critical contributors to disease. To focus the subsequent studies, single mutants were tested, and a *sae* mutant caused less bone destruction and a significant decrease in *S. aureus* burden as compared to an *agr* mutant. To confirm these observations and delve deeper into mechanism, *in vitro* cell-culture assays of osteoblasts indicated that indeed the *sae* mutant displayed a less virulent phenotype. Further, proteomic approaches were used to investigate exoproteome changes and uncovered 49 proteins down-regulated and 31 proteins up-regulated in the *sae* mutant. Interestingly, the bacterial metalloprotease aureolysin was most enriched in abundance, a fact supported by previous regulatory studies on *sae* mutants.

Building on previous reports on how aureolysin modulates pathogenic capacity (Zielinska et al., 2011), Cassat *et al.* (2013) hypothesized that aureolysin functions as the primary switch responsible for inducing bone remodeling during infection by tailoring the *S. aureus* secreted virulence factor arsenal. Aureolysin is a calcium and zinc-dependent metalloenzyme and one of the four major extracellular proteases secreted by *S. aureus* strains. The enzyme self-activates upon secretion, preferentially cleaves on the amino-terminal side of branched-chain amino acids, and serves as the starting point of the proteolytic activation cascade. The authors (Cassat et al., 2013) compared exoproteome pools to identify proteins that were aureolysin labile. One group of proteins that stood out were the alpha-type phenol-soluble modulins (PSMα), which is a collection of four low MW peptide toxins with known functions in neutrophil killing and infection. Assessment of a *S. aureus* strain lacking PSMα1–4 indicated this mutant had pronounced defects in osteoblast killing as well as significant attenuation in bone destruction and new bone growth in the murine model. Follow-up studies relying on exogenous peptide addition demonstrated that chemically synthesized PSMα2 was the most cytotoxic to osteoblasts, whereas PSMα4 was relatively non-toxic. Pre-treatment of PSMα peptides with aureolysin greatly diminished their cytotoxic properties to osteoblasts, supporting previous studies that these peptides serve as aureolysin substrates (Gonzalez et al., 2012). Thus, by controlling the extracellular concentration of one enzyme, *S. aureus* is able to modulate the potency of its own secreted proteome and dictate the course of osteomyelitis and potentially other infections. While the authors focused on the contribution of PSMα peptides to osteomyelitis, proteomic analysis demonstrated numerous other factors are influenced by aureolysin levels, indicating that the exoproteome is a dynamic entity and aureolysin functions as a rheostat to modulate pathogenic capacity.

Going beyond this study, the development of the new microCT imaging platform has the potential to assess osteomyelitis with other pathogens or related bone infections. A number of other bacterial pathogens cause osteomyelitis, including *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Acinetobacter baumanni*, *Pseudomonas aeruginosa*, and *Escherichia coli*, and one could easily imagine applying this murine model and microCT approach to monitoring disease progression and follow-up mechanistic analysis with any of these pathogens. Additionally, as the author's state (Cassat et al., 2013), the work could

serve as means to test chemo- or immunotherapeutic approaches to osteomyelitis treatments, taking advantage of the imaging platform as a means of monitoring and quantifying bone damage and regrowth. There is also potential to apply the murine model and microCT approach to other related types of bone infections. *S. aureus* is a leading cause of prosthetic-joint infections (Kourbatova et al., 2005, Zimmerli et al., 2004), and the numbers of patients getting joint replacements is increasing. Infection rates following the initial replacement are usually low, only 1–2%, but increase substantially in revision surgery (Lentino, 2003). When an infection occurs, it can be a devastating complication having an associated high morbidity and substantial financial burden. Extending development of the microCT platform, it may be possible to assess disease progression in the presence of the implanted materials, allowing additional mechanisms of pathogenesis to be deciphered in this context. Going further, there is special consideration in orthopaedic surgery for the high rate of infections in osteoarticular allograft reconstructions. These reconstructions permit reestablishment of skeletal continuity and function after a wide resection of bone tumor, and allografts are increasingly used in salvage of difficult bone stock deficiencies following failed total joint replacements (Muscolo et al., 2005). However, allograft infection is a disastrous complication as it may lead to limb amputation (Mankin et al., 2005), and again *S. aureus* is one of the most common causes. A better understanding of the mechanism underlying these infections through further development of the murine model and microCT platform could greatly improve the outcomes of surgical reconstructions.

In recent years, many exciting advances in infection imaging have been pioneered by researchers that may eventually aid a physician's assessment and treatment of certain diseases. The microCT imaging modality developed in this work is another impressive example that can be added to the expanding toolbox of approaches for monitoring disease course. Further development and translation of this technology has great potential to improve the disease prognosis for osteomyelitis patients by both facilitating the ability to track infection progression and aiding in the discovery and testing of innovative therapeutic interventions. Presently, this technology can be applied to enhance our knowledge of *Staphylococcus aureus* mechanisms of osteomyelitis and potentially extend to other bacterial pathogens as well. With the knowledge gained from imaging of this murine model, therapies that limit osteomyelitis-dependent bone destruction and enhance antimicrobial efficacy could eventually lead to improved patient outcomes for this devastating disease.

References

Cassat JE, Hammer ND, Campbell JP, Benson MA, Perrien DS, Mrak LN, Smeltzer MS, Torres VJ, Skaar EP. A secreted protease tailors the *Staphylococcus aureus* virulence repertoire to modulate bone remodeling during osteomyelitis. Cell Host & Microbe. 2013 this issue.

Gonzalez DJ, Okumura CY, Hollands A, Kersten R, Akong-Moore K, Pence MA, Malone CL, Derieux J, Moore BS, Horswill AR, Dixon JE, Dorrestein PC, Nizet V. Novel phenol-soluble modulin derivatives in community-associated methicillin-resistant *Staphylococcus aureus* identified through imaging mass spectrometry. J Biol Chem. 2012; 287:13889–13898. [PubMed: 22371493]

Hatzenbuehler J, Pulling TJ. Diagnosis and management of osteomyelitis. Am Fam Physician. 2011; 84:1027–1033. [PubMed: 22046943]

Kourbatova EV, Halvosa JS, King MD, Ray SM, White N, Blumberg HM. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA 300 clone as a cause of health care-associated infections among patients with prosthetic joint infections. Am J Infect Control. 2005; 33:385–391. [PubMed: 16153484]

Lentino JR. Prosthetic joint infections: bane of orthopedists, challenge for infectious disease specialists. Clin Infect Dis. 2003; 36:1157–1161. [PubMed: 12715311]

Lew DP, Waldvogel FA. Osteomyelitis. Lancet. 2004; 364:369–379. [PubMed: 15276398]

Mankin HJ, Hornicek FJ, Raskin KA. Infection in massive bone allografts. Clin Orthop Relat Res. 2005:210–216. [PubMed: 15738824]

Muscolo DL, Ayerza MA, Aponte-Tinao LA, Ranalletta M. Use of distal femoral osteoarticular allografts in limb salvage surgery. J Bone Joint Surg Am. 2005; 87:2449–2455. [PubMed: 16264120]

Zielinska AK, Beenken KE, Joo HS, Mrak LN, Griffin LM, Luong TT, Lee CY, Otto M, Shaw LN, Smeltzer MS. Defining the strain-dependent impact of the Staphylococcal accessory regulator (*sarA*) on the alpha-toxin phenotype of *Staphylococcus aureus*. J Bacteriol. 2011; 193:2948–2958. [PubMed: 21478342]

Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med. 2004; 351:1645–1654. [PubMed: 15483283]

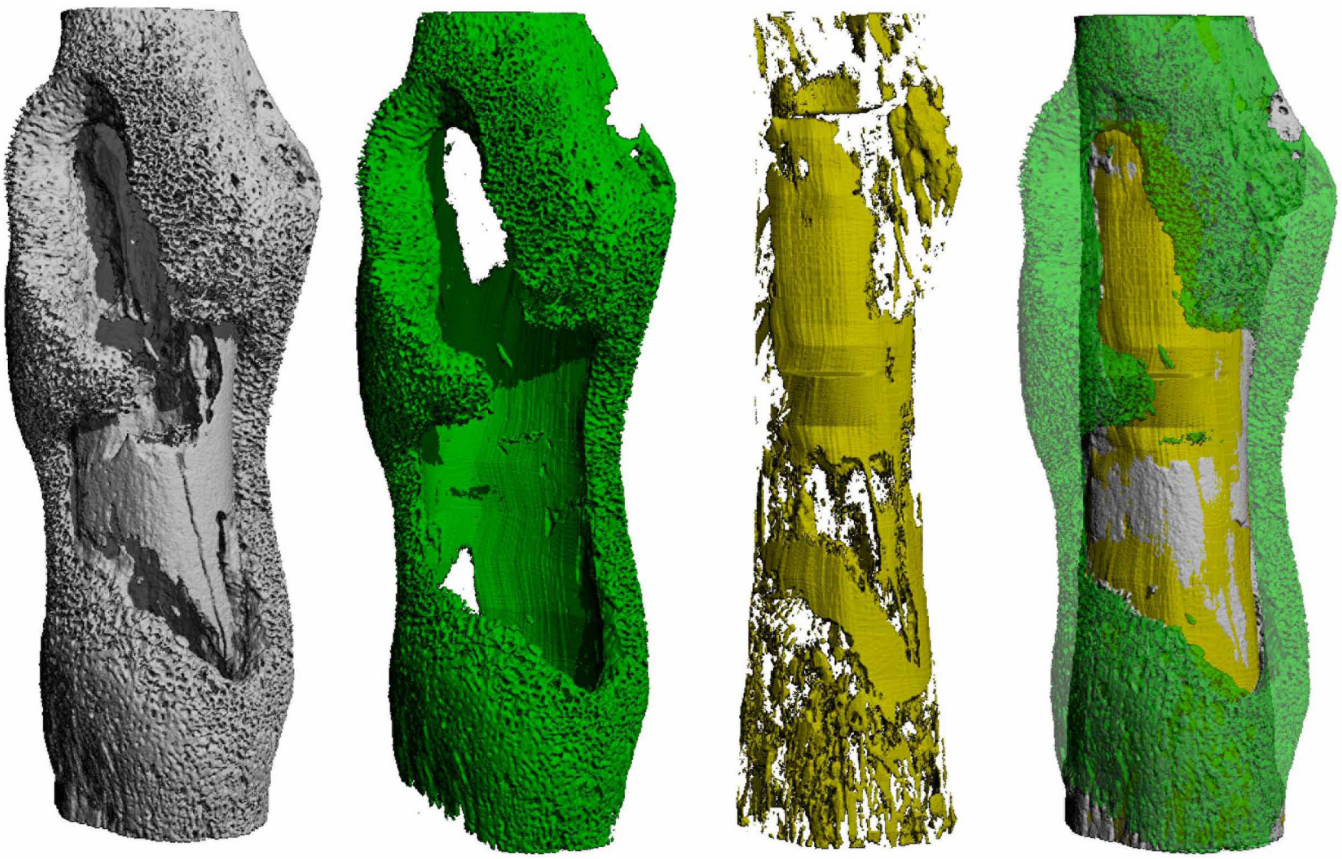

Figure 1. Imaging *S. aureus*-induced pathologic bone remodeling during osteomyelitis

S. aureus triggers profound alterations in bone remodeling during osteomyelitis (left image). Cassat et al. (2013) created imaging analysis algorithms to precisely quantify pathologic new bone formation (green) and cortical bone destruction (yellow), enabling comparison of the destructive capacity of different staphylococcal strains. Image provided courtesy of Dr. James Cassat, Vanderbilt University School of Medicine, USA.