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Mechanisms of Immune Evasion and Bone Tissue Colonization That Make *Staphylococcus aureus* the Primary Pathogen in Osteomyelitis

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Abstract

Purpose of Review *Staphylococcus aureus* is the primary pathogen responsible for osteomyelitis, which remains a major healthcare burden. To understand its dominance, here we review the unique pathogenic mechanisms utilized by *S. aureus* that enable it to cause incurable osteomyelitis.

Recent Findings Using an arsenal of toxins and virulence proteins, *S. aureus* kills and usurps immune cells during infection, to produce non-neutralizing pathogenic antibodies that thwart adaptive immunity. *S. aureus* also has specific mechanisms for distinct biofilm formation on implants, necrotic bone tissue, bone marrow, and within the osteocyte lacuno-canicular networks (OLCN) of live bone. In vitro studies have also demonstrated potential for intracellular colonization of osteocytes, osteoblasts, and osteoclasts.

Summary *S. aureus* has evolved a multitude of virulence mechanisms to achieve life-long infection of the bone, most notably colonization of OLCN. Targeting *S. aureus* proteins involved in these pathways could provide new targets for antibiotics and immunotherapies.

Keywords *Staphylococcus aureus* · Osteomyelitis · Adaptive immunity · Immune proteome · Orthopedic infections · Canalicular invasion

Introduction

Hard-to-treat, deep infections such as osteomyelitis remain a significant healthcare problem in the USA and around the world [1]. Even with major advances in surgical procedures, and novel antimicrobial therapies, the treatment failure rate remains high [2–4]. The cost to treat prosthetic joint associated osteomyelitis is projected to rise to $1.62 billion a year by 2020 due to the aging population in the USA [1, 5, 6]. Unfortunately, there has been little or no reduction in infection-related outcomes in several decades as the current standard of care treatments, developed in the 1970s, are still being employed in the US hospitals [7•, 8•, 9•].

Osteomyelitis, defined as an infection of the bone, can occur by (1) the local spread of the bacteria from a contaminated bone to an adjacent uninfected bone, (2) contamination of the bone via the hematogenous route, and (3) bacterial invasion of the bone from an infected implant [10, 11]. There are five distinct classes of osteomyelitis. These include (1) prosthetic joint infection (PJI), the post-operative infection that occurs after a prosthetic joint has been placed, typically in the knee or hip; (2) fracture-related infection (FRI), a common complication in trauma surgery where patients typically present with open fractures; (3) acute hematogenous osteomyelitis (AHO), that arises due to seeding bacteria circulating in blood to the long bones; (4) diabetic foot infections (DFI), frequently observed as a polymicrobial infection of a diabetic foot ulcer that spreads contiguously to the underlying bone; and (5) osteomyelitis of the spine (OMS), that usually occurs postoperatively [1, 10, 11, 12•, 13, 14].

The incidence rate of all classes of orthopaedic infections range from 0.1–30%, and treatment can cost up to $150,000 per patient in the USA [7•]. The rate of PJI in primary arthroplasty remains between 1 and 2%, though reinfection

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rates remain far higher for these patients: 8% for hips and knees. Unfortunately, due to the aging population in the USA, and the increased number of elective total knee and hip arthroplasties in the last two decades, the anticipated incidence rate of PJI will likely increase in the near future. Moreover, there is increasing incidence of arthroplasty procedures for other joints such as the ankle and shoulder that could also affect the overall incidence of PJI [1, 6, 15]. The incidence rate for FRIs is much higher, from 10 to 50% depending on the fracture type [12, 16]. Typically, monomicrobial AHO represents about 20% of all osteomyelitis cases and is most prevalent in children and related to rapid bone growth; about 85% of AHO patients are under 17 years of age [17–19]. Recently, AHO rates have increased in adults due to secondary conditions such as bacteremia induced by intravenous drug abuse associated with the opioid abuse crisis in the USA [20, 21]. Among the osteomyelitis patients, DFI patients have the highest mortality risk. Their 5-year mortality rate is reported to be 50%, equal to that of the most life-threatening cancers [22]. Moreover, their quality of life is among the poorest of the osteomyelitis patients as two thirds of lower extremity amputations are associated with DFI [13, 23, 24]. Finally, the post-operative occurrence rate of OMS is between 0.3 and 20%, a prevalence rate that is higher than all of the other classes of orthopedic infections [14].

Most research on bone infections has centered on *Staphylococcus aureus* (*S. aureus*) due to its frequency, plasticity, and resistance, and because it causes the majority of osteomyelitis cases. *S. aureus* is a gram-positive coccus, first isolated by Alexander Ogston from the pus of surgical wound infections in the1880s. An astounding 50% of the prosthetic joint-related orthopedic infections are caused by difficult-to-treat MRSA strains [15, 25–27]. It is a successful pathogen that has evolved to infect nearly every organ system of the human body through its vast immune evasion and persistence mechanisms. In the context of osteomyelitis, *S. aureus* harnesses these mechanisms to persist within various tissue types and, in doing so, alters its state of growth to infect for years or even decades [28–31]. There is an urgent need to control *S. aureus* osteomyelitis. To achieve that goal, we need a better understanding of the intricate immune evasion mechanisms that the pathogen employees to successfully invade and thrive in the bone environment. In this review, we will summarize these mechanisms with a particular focus on the host's adaptive immunity and *S. aureus*' mechanisms for circumventing it.

Adaptive Immune Responses in Host Defense to *S. aureus* Osteomyelitis

Adaptive immunity against *S. aureus* osteomyelitis consists of cell-mediated immune responses dominated by T cells and humoral antibody responses mediated by B cells. Adaptive immune responses are triggered after a week of *S. aureus* infection. These typically occur after presentation of antigens to dendritic cells and subsequent activation of T cells. Our understanding of the role of T cells in *S. aureus* infections have vastly improved over the past 20 years (reviewed elsewhere [32••, 33, 34]). Activated T cells, subsequently activate B cells that differentiate into plasma cells, the producers of antigen-specific antibodies. A portion of these activated B cells become memory cells that can be recalled to produce antibodies during reinfections. Unfortunately, because *S. aureus* can cause persistent and chronic infections, such as osteomyelitis, adaptive memory responses are not entirely effective. In this review, we will focus on B cell response mechanisms and how *S. aureus* cleverly evades humoral immune responses during chronic osteomyelitis. Specifically, we will discuss how *S. aureus* manipulates B cell function and survival during infection. We will also discuss studies that focus on *S. aureus* humoral immune proteome, the sum of all the host's antibodies produced against the pathogen.

Manipulation of B Cells by *S. aureus*

The ability of *S. aureus* to cause disease is largely attributed to the expression of its vast array of virulence factors including immunomodulatory proteins, adhesins, toxins, and superantigens, several of which have redundant functions. *S. aureus* manipulates B cell survival and function through the production of staphylococcal protein A (SpA), a sortase-anchored protein with very high affinity to human immunoglobulins. The immunomodulatory effects of SpA have been attributed to two distinct binding activities: association with (1) the Fc domains of most human IgG molecules and (2) the Fab domains of certain antibody variable region families [35–37]. During infection, SpA is released into host tissues where it binds to the Fcγ domain of IgG, blocking antibody-mediated phagocytosis. SpA is also capable of binding the Fab domains to crosslink the V_H3 chain of IgM antibodies. This in turn, causes proliferative expansion of B cells, which ultimately leads to their collapse by apoptosis [38, 39]. Interestingly, Pauli and colleagues demonstrated that activated B cells, during infection, elicit a highly limited response with a significant bias towards V_H3 idiotype. They also found that maturing plasmablasts had high affinity to SpA [40]. Limiting the host's B cell response predominantly to a particular immunodominant antigen such as SpA is one-way *S. aureus* ensures that there is no protection or memory against other virulence proteins during a chronic infection like osteomyelitis. A recent study also demonstrated that SpA reduced the pool of bone marrow (BM)-resident long-lived plasma cells that are responsible for secreting protective antibodies [41]. Indeed, SpA variants that cannot bind to immunoglobulins demonstrated attenuated disease in a murine model of bacteremia. It was shown that the adaptive immune response in these mice produced antibodies against many antigens that

were protective against recurrent infections [40, 42•]. Currently, a non-toxogenic variant of SpA is being actively pursued as a passive and active vaccine candidate against *S. aureus* colonization and chronic infections [43–46].

To better understand the interaction of Staphylococci with human B cells, Nygaard and colleagues performed B cell association studies with *S. aureus* and *Staphylococcus epidermidis*. They observed that a significantly larger proportion of B cells associated with *S. epidermidis* than with *S. aureus* and the observed binding was mediated by abundant proteins in the complement cascade [47]. This study highlighted the importance of pathogen-produced virulence molecules for the inhibition of the complement pathway and complement-mediated opsonophagocytosis. Staphylococcal complement inhibitor (SCIN) and extracellular fibrinogen-binding protein (Efb) are secreted by *S. aureus* and inhibit the deposition of activated C3 and C4 derivatives on the bacteria's surface [48]. On a related note, we have demonstrated that anti-SCIN and anti-Efb IgG levels in human serum can be useful diagnostic markers to identify *S. aureus* osteomyelitis in patients [49••].

Humoral Immune Proteome of *S. aureus* During Osteomyelitis

Due to life-long exposure to *S. aureus* through prior infections or asymptomatic colonization, anti-*S. aureus* antibodies are prevalent in all humans after only a few weeks of age [50–54]. The sum total of all of the host's antibodies against a particular pathogen is termed the "humoral immune proteome." Unfortunately, as mentioned, the presence of these pre-existing antibodies does not confer protection against *S. aureus* reinfection or persistent infections [50–54]. This is because not all pre-existing antibodies are protective antibodies that can neutralize *S. aureus* virulence proteins and toxins and/or mediate phagocytic killing and clearance of the pathogen. In fact, there are cases where non-neutralizing or pathogenic antibodies can facilitate the growth and dissemination of *S. aureus*.

Even though the antibodies produced are limited in their host protection, the anti-*S. aureus* humoral immune response in physiologic and pathologic conditions can be a useful tool for the diagnosis of *S. aureus* infections, especially for deep infections such as osteomyelitis. These infections are difficult to identify, and patients typically present with non-specific symptoms such as pain, fever, and swelling of the joint. Utilizing a multi-antigen Luminex immunoassay, we defined the humoral immune proteome of patients with *S. aureus* osteomyelitis. Iron-scavenging proteins (IsdA, IsdB), and cell wall enzyme bifunctional autolysin (amidase (Amd) and glucosaminidase (Gmd)) were the most immunodominant antigens during *S. aureus* osteomyelitis [49••]. Among these antigens, we observed that certain ones could be predictors of outcomes of *S. aureus* infections in patients. Specifically, higher serum anti-IsdA and anti-IsdB IgG levels correlated with increased mortality in patients, indicating their role as pathogenic antibodies, while anti-Gmd IgG levels correlated with protection against *S. aureus* osteomyelitis, identifying these as protective antibodies [49••, 55]. Indeed, we predict morbid outcomes if antigens that elicit pathogenic antibodies would ever be used as potential vaccine candidates. A case in point was the phase 2/3 clinical trial of an IsdB active vaccine (Merck's V710) [56]. This 8000-patient clinical trial was abruptly terminated because of a fivefold increase in patient mortality induced by multiple organ sepsis in the IsdB-vaccinated group. We hypothesize that non-neutralizing pathogenic IsdB antibodies, generated by vaccination, promoted *S. aureus* growth and dissemination into distal organs. In a murine model of *S. aureus* osteomyelitis, we observed that active and passive IsdB immunizations rendered mice more susceptible to multiple organ sepsis (Nishitani, Ishikawa, Schwarz (unpublished results)). In sharp contrast to non-neutralizing pathogenic IsdB antibodies, neutralizing anti-Gmd monoclonal antibody significantly reduced MRSA infection severity in a murine model of implant-associated osteomyelitis [57, 58•]. Our laboratory is currently pursuing anti-Gmd passive immunization strategies to prevent and treat *S. aureus* osteomyelitis. Assessment of neutralizing antibodies and humoral immune responses against acute *S. aureus* infections, such as bacteremia and skin and soft tissue infections, is being pursued by other investigators [59–61].

In addition to antibody-producing long-lived plasma cells, newly activated pathogen-specific B cells called circulating plasmablasts or antibody-secreting cells (ASC) are of key importance for protecting against *S. aureus* infections. These ASC are activated early in an infection and disseminate into the blood en route to other lymphoid tissues as long as the infecting pathogen remains active [62•, 63–65]. It is unclear if these recently activated plasmablasts produce neutralizing or pathogenic antibodies. Nonetheless, the antibodies produced by these cells can be useful diagnostic and prognostic markers of ongoing bacterial and viral infections [63, 64, 66, 67, 68••]. In a recent study involving chronic osteomyelitis in patients that were undergoing foot salvage therapy (FST) for diabetic foot infections (DFI), we demonstrated that antibodies secreted by plasmablasts can not only accurately diagnose ongoing infections, but can also track treatment responses and detect persistent or recurrent infections that are indiscernible in serum response [68••]. Thus, measuring the humoral immune proteome in a simple immunoassay could significantly change the way *S. aureus* osteomyelitis is diagnosed and treated.

Immune Evasion Strategies and *S. aureus* Persistence in Osteomyelitis

Bacterial infection of the bone presents a unique set of immune challenges because of the different modes of colonization and persistence within the bone. Here we consider four

immune mechanisms of *S. aureus* persistence in the context of osteomyelitis: (1) abscesses; (2) biofilms; (3) invasion of the osteocyte lacuno-canalicular network (OLCN) of bone; and (4) intracellular infection (Fig. 1). In the short term, bacteria can evade innate immune attack and survive within abscesses located in the bone marrow and the surrounding soft tissue. As acute bone infection progresses, the local tissue becomes devascularized and sequestered to isolate the infected region from healthy tissue. The sequestered tissue becomes a haven for bacterial persistence in the form of biofilms and within the OLCN of bone. Finally, the possibility of intracellular infection has been investigated recently as an additional mechanism of persistence in osteomyelitis. Together, these adaptive defense mechanisms allow *S. aureus* to chronically infect the bone despite all efforts of eradication.

Abscess Formation

Abscess formation is typically considered a host-induced mechanism of infection control initiated by innate immune cells to sequester infected tissue and associated pathogens. However, it is known that *S. aureus* manipulates the host response by deploying an array of virulence factors that trigger the formation of the multilayered structure of an abscess with protected bacterial cells at its core. Described as "a play in four acts" [69], *S. aureus* must first overcome innate immune defenses within the bloodstream, including phagocytes, complement, and antimicrobial peptides (AMPs). This is accomplished via expression of microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) including clumping factor A (ClfA) [70] that facilitates binding to

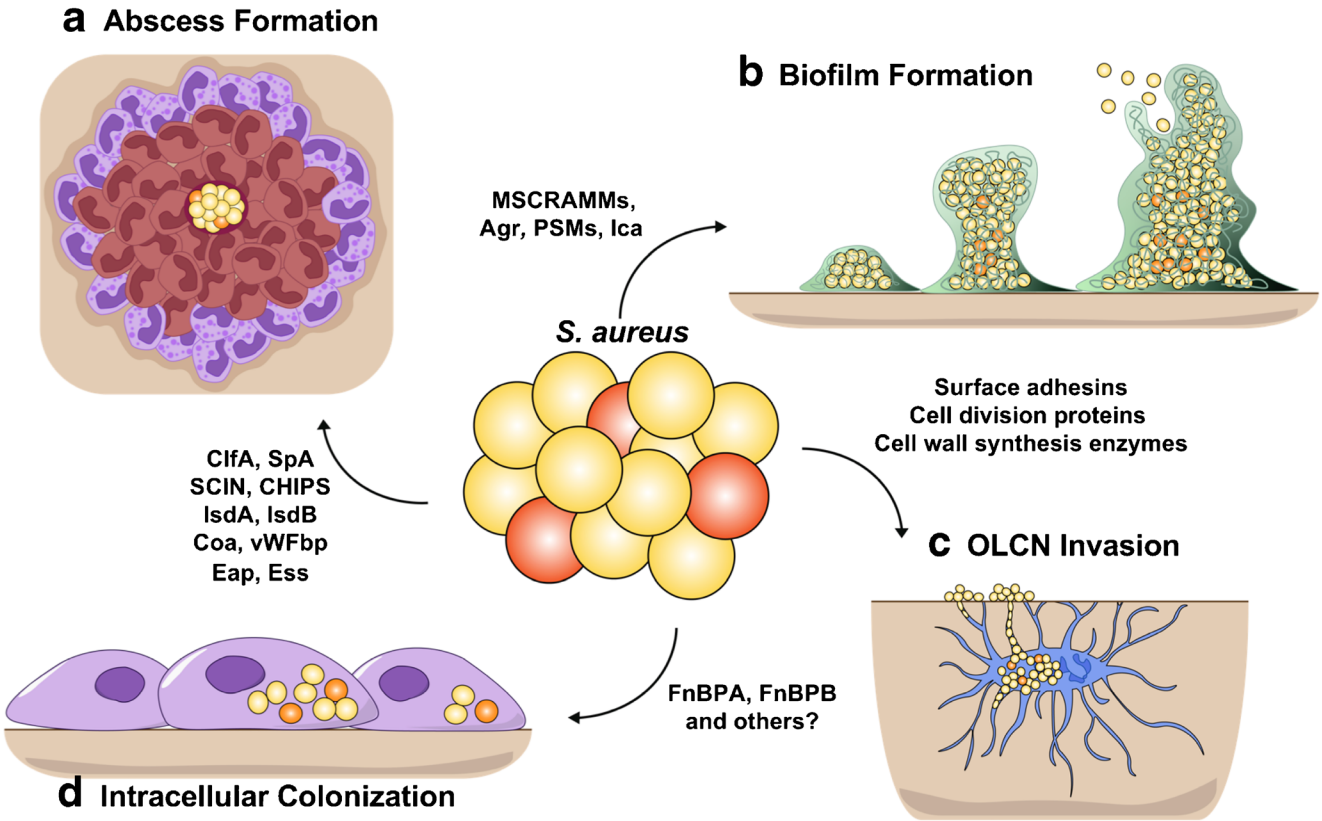

Fig. 1 "Four acts" of immune evasion and bacterial persistence during *S. aureus* osteomyelitis. *S. aureus* in the bone environment is presented with several immune challenges. Four important immune mechanisms of *S. aureus* persistence in the context of osteomyelitis are as follows: (1) abscess formation; (2) biofilm formation; (3) invasion of the osteocyte lacuno-canalicular network (OLCN) of the bone; and (4) intracellular colonization. (A) *S. aureus* hijacks the host-induced abscess formation process to create an abscess with protected bacterial cells at its core, which are inaccessible to immune cell infiltration. *S. aureus* deploys numerous cell wall-associated and secreted virulence proteins to trigger the formation of this multilayered structure. (B) Necrotic bone or metal implants are ideally suited for *S. aureus* biofilm formation. As illustrated, the process of biofilm development involves bacterial colonization/attachment, maturation, detachment, and dissemination. Dozens of MSCRAMMs and virulence molecules such as Ica, PSMs, play critical roles in this process. These factors are regulated by *agr* quorum sensing system. (C) A recently uncovered mechanism of chronic bacterial persistence is the invasion of the submicron channels deep within the cortical bone. Electron microscopy analyses of infected necrotic bone revealed the presence of *S. aureus*, which usually is a micron in diameter, in these submicron channels. Several surface adhesins, cell wall synthesis proteins, and cell division enzymes are hypothesized, but not yet confirmed, to play significant roles in OCLN invasion. (D) Several in vitro, but not in vivo, studies have also demonstrated that *S. aureus* can survive intracellularly in non-professional phagocytes such as osteoblasts, osteoclasts, and osteocytes during osteomyelitis. Specific proteins involved in this process of internalization have yet to be fully deciphered. It is also believed that in all the aforementioned mechanisms, *S. aureus* can also transform into a quasi-dormant small colony variant (SCV, illustrated as red bacteria) and persist in the bone for long periods of time.

fibrin or fibrinogen and results in aggregation of *S. aureus* colonies that are resistant to phagocytosis [71].

In addition to cell wall-associated enzymes and binding molecules, *S. aureus* secretes proteins such as chemotaxis inhibitory protein of staphylococci (CHIPS) [72] and SCIN [73] to evade the early-stage acute immune response. Following distribution to various organ sites via the bloodstream, the pathogen recruits large numbers of neutrophils and other innate immune cells to the site of infection. The expression of proinflammatory iron-scavenging proteins IsdB, IsdA, and IsdH promotes the local secretion of inflammatory cytokines, triggering an influx of neutrophils [74, 75]. The third stage of staphylococcal abscess maturation involves the formation of a multilayered structure of dead and live neutrophils around a central core of live *S. aureus* cells [76]. Expression of coagulase (Coa), as well as von Willebrand's factor-binding protein (vWFbp), are key to this stage of abscess maturation as they assist in the formation of a protective fibrin "pseudocapsule" [77]. Other genes required for abscess formation and maturation appear to be SpA and Emp since their mutants are unable to form proper abscesses [76]. The fourth and final stage of staphylococcal abscess maturation is steady persistence, followed by the ultimate bacterial egress and dissemination to new sites. It has been shown that *S. aureus* mutants lacking the immunomodulatory proteins Eap and Ess are deficient in abscess persistence, suggesting their involvement in prolonged survival within an abscess [78].

Staphylococcal abscesses during osteomyelitis are usually formed in the bone marrow space as well as in adjacent soft tissues [58, 79]. In a mouse model of implant-associated osteomyelitis, it was shown that diabetic obese mice had more severe infections with an increased number of bone abscesses relative to healthy control mice [80]. Further, *S. aureus* isolated from type-2-diabetic infections exhibited upregulation of genes associated with fibrin and fibrinogen binding [80].

Biofilm Formation

Staphylococcal biofilms formed on implanted materials and necrotic bone are the most recognized forms of *S. aureus* persistence in osteomyelitis. In orthopedic infections, biofilms are presumed to be the leading cause of incurable, chronic infections [81–83]. The formation of a biofilm begins with the initial attachment of planktonic bacterial cells to a substrate. This attachment step is mediated by *S. aureus* MSCRAMMs that have the ability to bind a wide array of extracellular matrix proteins. The attachment of planktonic cells then triggers the synthesis of extracellular polymeric substances (EPS) including polysaccharides, proteins, and nucleic acids. Together, these substances encase the bacterial cells, prevent immune cell attack, mediate adhesion, provide mechanical stability, and retain essential nutrients and enzymes [84].

When embedded within the protective EPS matrix, bacterial cells can survive drug dosing of up to 1000 times greater than their planktonic phenotype [85]. The elevated resistance to antibiotics in the biofilm phenotype is, in part, due to decreased drug diffusion from the exterior of the biofilm to the bacteria that are buried deeply within. Additionally, the effectiveness of antibiotics that successfully diffuse through the biofilm is diminished due to the altered metabolic phenotype of bacteria growing in biofilms, making them more resistant to drug treatments.

As the bacteria continue to divide within the biofilm, local nutrients become depleted, and additional survival strategies are deployed. First, some bacteria within the biofilm undergo phenotypic or genotypic changes that dramatically reduce their metabolic requirements [86, 87]. In doing so, a subpopulation of the bacteria shifts into a slow-growing persistent state. Because of these changes, the slow-growing bacterial population is also more resistant to antibiotics that attack actively dividing bacteria [86, 87]. A second *S. aureus* survival strategy is the activation of the accessory gene regulator (*agr*) quorum sensing system [88]. The *agr* quorum sensing system is commonly considered to be the primary regulator of virulence factors in *S. aureus* as its activation triggers the expression of secreted toxins such as α-hemolysin (Hla) and the phenol soluble modulins (PSMs) [89]. Other virulence factors that play a critical role in biofilm formation during chronic osteomyelitis are discussed elsewhere [90, 91].

Unfortunately, to date, there is no standard method for in vitro biofilm growth, making comparison of results from lab-to-lab and hospital-to-hospital widely variable [92]. Variations in growth media, culture time, and presence of shear flow result in very different biofilm models, which drastically impact the results of a study. Several in vitro biofilm growth models have been developed to improve the clinical relevance of biofilms such as capillary flow cell bioreactors [93, 94], the Calgary Biofilm Device [95], microcarrier pebbles [92], or supplemented media [96]. However, there is no consensus yet on how to properly culture *S. aureus* biofilms. In our estimation, mouse models of implant-associated osteomyelitis are the most effective methods to mimic the in vivo biofilms that occur in the clinic [97, 98].

Osteocyte Lacuno-Canalicular Network Invasion

A newly discovered mechanism of immune evasion and persistence in chronic osteomyelitis is the invasion of the submicron channels deep within the cortical bone. Buried within the dense mineral matrix of bone are osteocytes, which are interconnected via canaliculi containing their cell processes. Their primary function is to maintain bone homeostasis by mechanotransduction [99]. In recent work, we have observed *S. aureus* invasion of the OLCN of bone by transmission electron microscopy [100••]. Originally discovered in mouse

models of implant-associated osteomyelitis, and later confirmed in a clinical case of diabetic foot infection, invasion of the OLCN appears to be a newly recognized mechanism of chronic bone infection [100••, 101••].

Staphylococcal invasion of the OLCN is particularly concerning for multiple reasons. First, *S. aureus* within the confined geometries of canaliculi or lacunae are completely protected from immune cell attack. Second, it is possible that the bacteria can survive for years by dissolving the surrounding bone mineral matrix as a source of nutrients. Finally, the depth of *S. aureus* invasion within the OLCN is not known but it could be a major factor in the failure of surgical debridement of infected bone.

It was shown that *S. aureus* is capable of colonizing and proliferating within the submicron canaliculi of bone, requiring them to deform as small as 0.2 μm in diameter. Further, BrdU immunoelectron microscopy was used to confirm that the bacterial cells at the leading edge of invasion are actively proliferating, as opposed to persisting in a dormant state. This finding was surprising given that *S. aureus* is a non-motile bacterium with no known mechanisms of active translocation. At this point, we hypothesized that *S. aureus* has a novel mechanism of invasion that enables asymmetric cell division into the opening of a canaliculus and onwards through the channel [100••].

In order to determine the mechanism of *S. aureus* invasion of the OLCN and to identify virulence genes responsible for haptotaxis and durotaxis, an in vitro model of canalicular invasion, named the μSiM-CA, has been developed [100••, 102]. Our recent work demonstrated that the *agr* quorum sensing system may not be necessary for propagation through 0.5 μm nanopores in vitro [102]. This result was confirmed in our mouse model of implant-associated osteomyelitis, where an *agr* deletion strain of *S. aureus* was capable of invading the submicron canalicular network of bone. Continued work using the μSiM-CA is warranted to determine the genetic mechanism of *S. aureus* invasion of the OLCN.

Intracellular Colonization of Non-professional Phagocytes

The final potential mechanism of *S. aureus* persistence in osteomyelitis is long-term intracellular infection. *S. aureus* is no longer defined as a strictly extracellular pathogen, and it is known that *S. aureus* can survive and proliferate within leukocytes during sepsis [79]. Numerous studies have documented internalization of *S. aureus* by non-professional phagocytes including epithelial cells [103], endothelial cells [104], keratinocytes [105], and fibroblasts [105] in a variety of infection settings. It is thought that *S. aureus* triggers internalization by FnBPA and FnBPB binding fibrinogen and bridging to $\alpha_5\beta_1$ integrins [106]. Following internalization, the bacteria can evade cell death via persistence within vacuoles, endosomal escape, or by preventing phagolysosomal fusion [107].

Specifically, in the context of osteomyelitis, osteoblasts [108–110], osteoclasts [111], and osteocytes [112] have been extensively studied in vitro as possible reservoirs for intracellular colonization. Intracellular persistence, even for a short window of time, may provide the bacteria with a mechanism of immune cell evasion as well as protection from antibiotic treatments. It is hypothesized that in order to persist intracellularly, *S. aureus* adopts a slow-growing, metabolically inactive state like that of small colony variants (SCVs) [113]. Unfortunately, the majority of these studies were performed in vitro with little relevance to the dynamic host-pathogen interactions in vivo. Currently, very few in vivo or clinical studies have linked *S. aureus* intracellular infection with chronic osteomyelitis [30, 87, 114]. Thus, some crucial questions that remain unanswered are the length of time *S. aureus* can persist intracellularly in bone cells and if intracellular *S. aureus* is a cause of recurrent osteomyelitis.

Conclusions

S. aureus osteomyelitis continues to be a major clinical problem and the bane of orthopedic surgery. Elucidation of several *S. aureus* immune evasion mechanisms and definition of its humoral immune proteome in osteomyelitis have markedly improved our understanding of chronic osteomyelitis over the past few decades. Moreover, the recent discovery of *S. aureus* invasion of the OLCN provides a clear explanation for why this form of bone infection is incurable. With this knowledge of the complex pathophysiology of *S. aureus* osteomyelitis, the current challenge is to translate this new information into novel tests and interventions to properly diagnose and treat the most common forms of bone infection.

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Compliance with Ethical Standards

Conflict of Interest Gowrishankar Muthukrishnan reports grants from AOTrauma Research Fellowship, during the conduct of the study. John Daiss reports grants from NIH NIAID and is the cofounder of Micro B-Plex and works there part-time, during the conduct of the study. Edward Schwarz reports grants from AO Trauma and is the founder of Telephus Medical LLC. Dr. Daiss and Dr. Schwarz also have a patent (antibody-based diagnostics of *S. aureus* osteomyelitis) pending.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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