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EDITORIAL COMMENTARY

Staphylococcus aureus Small Colony Variants: Difficult to Diagnose and Difficult to Treat

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(See the article by Sendi et al. on pages 961–7)

The article by Sendi et al. [1] describes 5 cases of prosthetic joint infection with small colony variants (SCVs) of *Staphylococcus aureus* for which a correct identification in the clinical microbiological laboratory was delayed because of the uncommon morphological and physiological properties of these organisms. Indeed, SCVs are characterized by a strong reduction in growth rate, an atypical colony morphology, and unusual biochemical characteristics, which causes them to be frequently undetected or misidentified by standard clinical microbiology procedures [2]. The difficult recovery and identification of SCVs derived from *S. aureus* or other bacterial species may also explain the relatively sparse number of clinical reports describing their involvement in persistent and recurrent infections [2]. The clinical and microbiological data presented by Sendi and colleagues suggest that all 5 orthopedic patients were initially infected with normally growing *S. aureus*, which was followed by the subsequent emergence of clonally related SCV derivatives of the primary pathogens. Of note, the repeatedly observed treatment failure for these prosthetic joint infections with standard antimicrobial therapeutic protocols was clearly linked with the emergence of *S. aureus* SCVs. These consistent, clustered observations suggest that SCV-associated infections are not exceptional events and that their true frequency may be underestimated. The authors acknowledged that a lack of awareness initially contributed to ineffective surgical revisions and antimicrobial treatments in at least 2 of their patients. They now recommend actively screening for SCVs when *S. aureus* prosthetic joint infections persist or relapse despite adequate standard antimicrobial therapy.

Although several genetic and metabolic defects may contribute to SCV emergence, a frequent, common characteristic of these slow-growing organisms is their auxotrophic phenotype—namely, their requirement for specific substances that may eventually restore their growth rate to the parental level [2]. The 2 most frequently described groups of metabolic defects in SCV clinical isolates are those that lead to deficient electron transport—thereby affecting oxidative phosphorylation and ATP generation—and those that impair thymidine biosynthesis [2]. Because these defects and the ensuing SCV phenotype are often reversible (for reasons not fully understood), this reversion in phenotype may lead to the observation in clinical situations of mixtures of SCVs and normally growing parental organisms. The mixture of parental and SCV organisms may compromize SCV isolation and identification because of rapid overgrowth of slow-growing forms by normally growing parental organisms. Although defining the precise genetic lesion(s) in SCVs is hard to achieve, presumptive identification of the defective metabolic pathway may be obtained by simple detection of the auxotrophic requirement(s) (e.g., hemin, menadione, and thymidine) contributing to the SCV phenotype, combined with assessment of their degree of relatedness to their parental strains by molecular typing methods. The lack of specific auxotrophic testing in the study by Sendi et al. [1] may well have contributed to delayed identification of SCVs in their patients.

Of particular relevance is the observation in vitro and in vivo that exposure to different classes of antibiotics frequently contributes to the selection of SCVs. Aside from an overall nonspecific decrease in antibiotic susceptibility that may occur in SCVs because of their severely reduced growth rate and metabolic processes, some more-specific mechanisms, such as aminoglycoside or trimethoprim-sulphamethoxazol (SXT) resistance, should be mentioned. The reduction in aminoglycoside

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susceptibility is caused by a decrease in membrane potential that is linked to the downregulation of the electron transport chain. The clinical correlate of this observation has been the frequent isolation of electron transport–defective *S. aureus* SCVs in orthopedic patients treated with gentamicin-containing beads, which seem to significantly promote selection of SCVs over normally growing *S. aureus* [2]. In another clinically relevant situation, the emergence of thymidine-dependent SCVs derived from normally growing *S. aureus* was observed in patients with cystic fibrosis after long-term SXT treatment [3]. The positive selection of thymidine-dependent SCVs is currently explained by the stimulated uptake of extracellular thymidine that bypasses the SXT-inhibited pathway [2]. Interestingly, SCV isolates from 2 patients in Sendi and colleagues' study were resistant to SXT without prior clinical exposure to these antimicrobials, although their thymidine auxotrophy could not be experimentally assessed.

An additional key property of SCVs is their increased ability to survive within nonprofessional phagocytes, such as epithelial cells, fibroblasts, osteoblasts, and endothelial cells [2]. *S. aureus* is not traditionally considered to be an intracellular pathogen; however, over the last decade, published evidence of SCVs' ability to survive intracellularly has increased, from a few sporadic reports to, at present, a myriad of model systems that report bacterial uptake and persistence within nonprofessional phagocytes. Most of these studies reported cytopathological effects of intracellular *S. aureus*, but only a few of these studies specifically addressed the underlying mechanisms of intracellular survival. Notable advances include the report that intracellular *S. aureus* may persist in epithelial cells and may serve as reservoirs for recurrent infections in cases of chronic rhinosinusitis [4]. One explanation for the improved intracellular survival of SCVs is their decreased overall metabolism and dampened production of cytotoxins, which may downregulate the induction of cell lysis or apoptosis [2]. Their intracellular location, combined with the lack of efficient bactericidal mechanisms in nonprofessional phagocytes, are assumed to protect SCVs from professional phagocytes and from antimicrobial agents whose action is mainly extracellular. In their report, Sendi et al. [1] showed the presence of numerous intracellular staphylococci within fibroblasts, as visualized by transmission electron microscopy in repeated biopsy samples from a single patient. Although the authors assume that these intracellular cocci truly represent SCVs on the basis of microbiological arguments that the same biopsy specimens yielded pure SCV cultures, these transmission electron microscopy–derived conclusions should be regarded as preliminary, because the quality of the micrographs does not allow for the differentiation of SCVs from normally growing bacteria, compared with previous reports [2, 5]. Despite these limitations, the clinical transmission electron microscopy observations of intracellular *S. aureus* reservoirs as potential sources of persistent and recurrent infection confirm the great versatility and fitness adjustment of *S. aureus* to escape the strong selective pressure of bactericidal antibiotics and phagocytic clearance mechanisms through intracellular location and a quiescent lifestyle.

Progress in understanding SCV infections has been recently aided by detailed studies of regulatory and phenotypic properties [2], as well as an appreciation of enhanced surface expression of fibronectin-binding proteins that favor cell invasion kinetics [6]. Of particular note is the fact that 80% of debilitating human osteomyelitis infections are attributed to *S. aureus* that can invade and persist within osteoblasts [7, 8]. Apart from the anatomical and physiological characteristics of bone, which make antibiotic treatment of these infections challenging, is the realization that, if *S. aureus* persists intracellularly for >12 h, bacteria acquire significantly altered susceptibilities to antibiotics [7]. Knowledge of the underlying molecular mechanisms that permit survival and intracellular persistence of *S. aureus* is largely unexplored and would appear to be an urgent priority.

Although SCVs were considered for decades to produce essentially subacute, antibiotic-resistant infections, there is now increasing evidence that this may be just 1 part of a normal life cycle of staphylococci, with frequent switches from slow-growing to normally growing cells and vice versa [2]. In addition to recent experimental studies that have revealed some unsuspected virulence properties of SCVs in animal models of septic arthritis and endocarditis [2], clinical observations by Sendi and colleagues of bacteremia and bacteremic seeding from 1 hip prosthesis to the contralateral side in 2 patients seem to support the concept of an infectious cycle with alternating subacute and acute phases. Eventual confirmation of this life cycle model may explain the great difficulty in eradicating serious *S. aureus* infections with antimicrobial therapy. Indeed, the available antibiotic options for treating SCVs alone or in mixtures with normally growing cells are very limited and are further compromised by a high risk of emergence of antibiotic resistance. To target SCVs, antimicrobial agents should be able to penetrate host cells that contain bacteria and exert optimal intracellular bactericidal activity, especially against slowly growing bacteria. Rifampin is the single antistaphylococcal agent considered to be effective for treating intracellular infections, but its use in monotherapy is compromised by rapid emergence of high-level resistance. The combined regimen of rifampin with a fluoroquinolone, as proposed by Sendi et al. [1], may be the only available option, but this choice seems inadequate for treating methicillin-resistant *S. aureus* isolates, which are generally resistant to fluoroquinolones. Accordingly, the development of more-elaborate in vitro assays to reliably predict the outcome of antimicrobial therapy against SCVs is urgently needed.

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