Correspondence

Biofilm-Like Aggregation of Staphylococcus epidermidis in Synovial Fluid

TO THE EDITOR—We read with interest the recent article by Dastgheyb et al, which reported Staphylococcus aureus biofilm-like aggregate formation in human synovial fluid exceeding that observed in growth medium or serum [1]. This provides insight into S. aureus joint infection pathogenesis. Prosthetic joint infection (PJI), a burden for individuals and the healthcare industry, is becoming more common owing to the increasing number of primary arthroplasty surgeries [2]. Understanding PJI pathogenesis will inform its prevention, diagnosis, and management. Staphylococci account for up to 60% of PJIs, with S. aureus and Staphylococcus epidermidis predominating [2, 3]. Small colony variants (SCVs), a slow-growing phenotype that can survive intracellularly, are found in one third of staphylococcal PJIs, especially chronic PJIAs [3–10].

Herein, we assessed growth and aggregation of S. epidermidis, including SCVs, in synovial fluid. We grew S. epidermidis in 1:1 bovine synovial fluid (ATGO Industries, Tyler, Texas)/saline or growth medium (tryptic soy broth). In addition to reference strain RP62A and IDRL-8873 (a poor biofilm former), 3 pairs of S. epidermidis isolates from 3 infected knee arthroplasties were studied: IDRL-8934 (normal colony phenotype) and IDRL-8934 (SCV), IDRL-8864 (normal colony phenotype) and IDRL-8866 (SCV), and IDRL-8849 (normal colony phenotype) and IDRL-8850 (SCV) [3].

Growth in synovial fluid and tryptic soy broth was assessed by quantitative polymerase chain reaction (qPCR) as described by Dastgheyb et al, except that the gene encoding 16S ribosomal RNA was quantified instead of hla [1]. qPCR showed growth in synovial fluid comparable to that in tryptic soy broth, indicating that synovial fluid does not inhibit S. epidermidis growth (Figure 1A–D) [1]. IDRL-8934 and IDRL-8866, both SCVs, had slower growth in synovial fluid, compared with the paired isolates with normal colony phenotypes.

To determine whether S. epidermidis forms aggregates in synovial fluid, we visually examined bacteria 20 minutes and 24 hours after addition of 10^7 colony-forming units/mL to 1 mL of tryptic soy broth or 1:1 synovial fluid/saline with subsequent incubation at 37°C under static conditions. While there was no change after 20 minutes (data not shown), after 24 hours, all study strains, including those that formed poor biofilms in tryptic soy broth [5], formed macroscopic clumps in synovial fluid, whereas no clumping was observed in tryptic soy broth (Figure 1F). The aggregation phenotype of S. epidermidis recapitulates that observed by Dastgheyb et al for S. aureus, although Dastgheyb et al observed aggregation in 20 minutes [1]. In our assay, S. aureus USA300 formed more-pronounced macroscopic clumps than did S. epidermidis after 24 hours (Figure 1F) but did not aggregate at 20 minutes. Differences in time to aggregation between the 2 studies may be explained by the synovial fluid types studied. Dastgheyb et al studied synovial fluid obtained from humans undergoing arthroplasty, whereas we studied synovial fluid aspirated from healthy bovines; there may be increased host-derived fibrin in the former, contributing to rapid bacterial aggregation.

In conclusion, growth and formation of biofilm-like aggregates of S. aureus in synovial fluid, as reported by Dastgheyb et al, generalizes to S. epidermidis.

NOTES

Financial support. This work was supported by the National Institutes of Health (grants R01 AR56647, R01 AP91954, and GM055252).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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References


TO THE EDITOR—We read with interest the letter by Perez and Patel, who reported that *Staphylococcus epidermidis* strains form macroscopic aggregates during growth in synovial fluid that resemble those that we recently observed when *Staphylococcus aureus* was grown or incubated in synovial fluid [1]. The observations that Perez and Patel made of *S. epidermidis* are very similar to those we made of *S. aureus* [1], inasmuch as these authors also found strong bacterial aggregation in synovial fluid and ruled out the possibility that it was caused by other factors such as blood contamination or pooling of the synovial fluid itself.

Figure 1. *Staphylococcus epidermidis* grows and shows aggregation in synovial fluid (SF). A–D, Reference strain RP62A, a strain that has been shown to be a poor biofilm former (IDRL-8873), 6 clinical *S. epidermidis* strains and *Staphylococcus aureus* USA300 strains were grown in tryptic soy broth (TSB) or SF, using an initial inoculum 10⁷ colony-forming units/mL, with growth assessed by quantitative polymerase chain reaction. Black denotes normal colony phenotypes, blue denotes small colony variants, and gray denotes *S. aureus*. All experiments were performed in triplicate; error bars represent standard deviations. E and F, Cultures of *S. epidermidis* and *S. aureus* were incubated in the indicated fluids for 24 hours and examined visually. Red font denotes strains previously shown to be poor biofilm formers in TSB.