INTRODUCTION: *Staphylococcus aureus* (*S. aureus*) is the single leading cause of osteomyelitis, a bacterial infection of bone that is characterized by progressive inflammatory destruction (osteolysis). It is estimated that 20-50% of these cases are due to MRSA. Although the overall rate of MRSA infection in patients undergoing primary total joint replacement (TJR) surgery is very low (~1%), the management of these infections is very challenging and requires a two-stage surgical procedure. Moreover, the reinfection rate for revision TJR due to MRSA is very high (40-50%). Thus, investigations of non-traditional approaches, like passive immunization, to decrease the reinfection rate are warranted. To this end, the goal of this study is to evaluate the effects of monoclonal antibodies (mAbs) against the glucosaminidase (Gmd) subunit of *S. aureus* autolysin (Atl), which is known to be an immuno-dominant antigen in animal infection models. Since Atl is essential for cell wall digestion during binary fission, and Gmd mutants are unable to divide, we hypothesized that effective anti-Gmd mAbs will inhibit *S. aureus* proliferation and/or survival in *vivo* by inhibiting cell division and forcing the microbes to grow as fused bacteria.

METHODS: Antibodies: ELISA and dot-blot screening of 33 candidate hybridomas revealed five clones (1C11, 1E12, 2D11, 3A8, 3H6) that produced IgG1 mAb with high affinity binding to recombinant Gmd. These hybridomas were grown in DMEM media containing 10% FBS, and mAbs were purified from the culture supernatant using Protein G sepharose and then concentrated and dialyzed to make 1 mg/ml stocks.

Growth Assay: The *S. aureus* strain Xen29 was grown in LB media for 12 hours to achieve a mid-log growth suspension. 100 CFU Xen29 was placed in each treatment well of a 96-well plate, supplemented with 50 μg/ml of antibody, and readings were taken at 490 nm every two hours. The same growth assay was then slightly modified to study the dose-dependent effect of the five mAbs on *S. aureus* growth. MOPC-21 was used as an irrelevant isotype control in all growth assays.

Scanning Electron Microscopy: Xen29 *S. aureus* was grown for 12 hours in LB media to achieve a mid-log growth suspension. For the treatment group, 10,000 CFU of Xen29 was incubated with mAb 1C11 for 1 hour, whereas the negative control was not treated with mAb. Samples were then plated onto round glass coverslips, fixed, dehydrated, and coated with gold for visualization by SEM.

RESULTS: Four of the five monoclonal antibodies against glucosaminidase inhibited growth of *S. aureus* in our in vitro growth assays, whereas the irrelevant IgG1 control MOPC-21 had no effect on *S. aureus* growth (Figure 1a). The effect was dose-dependent and consistent with a high affinity interaction between each antibody and Gmd (Figure 1b). Gross observation of the LB liquid cultures revealed that the 1C11 precipitates Xen29 out of the growth media (Figure 2). Consistent with these observations and our hypothesis of Gmd inhibition, SEM revealed that *S. aureus* treated with 1C11 failed to complete binary fission and grew as a chain of bacteria in contrast to the single-cell suspension of the control culture (Figure 3). Collectively, these data indicate that anti-Gmd mAbs inhibit the growth of *S. aureus in vitro* by preventing cell division and planktonic growth.

DISCUSSION: The long-term goal of this program is the development of a passive immunization for MRSA. Our pursuit of anti-Gmd mAbs for this purpose is multifold. First, we originally identified Gmd as a protective immuno-dominant antigen in a murine model of implant osteomyelitis. Secondly, the DNA sequence of Gmd is 100% conserved in all known *S. aureus* strains, suggesting that mutation towards antigenic variation of this critical enzyme may not be possible. Lastly, we propose three distinct mechanisms of action for anti-Gmd mAbs that are synergistic with standard antibiotic chemotherapy: 1) opsonization to facilitate bacterial clearance by phagocytic cells; 2) complement mediated lysis of opsonized bacteria with exposed periplasm due to aborted cell wall metabolism; and 3) direct effects on bacterial viability and/or growth inhibition. Using Xen29 *S. aureus* as a model, we evaluated five high affinity anti-Gmd mAbs for their ability to disrupt critical steps during planktonic growth. In our growth assay experiments we demonstrated that anti-Gmd mAbs significantly inhibit *S. aureus* growth as determined by light scattering. Results from our ongoing metabolic labeling experiments to confirm this will be discussed. Additionally, scanning electron microscopy of treated cultures revealed that *S. aureus* failed to complete cell division, which explains the sedimentation observed in our treated cultures. These data indicate that anti-Gmd mAbs are attractive candidates for the development of a passive vaccine, and may prevent reinfection in patients undergoing total joint replacement revision surgery due to MRSA-induced osteomyelitis.