INTRODUCTION: Methicillin-resistant Staphylococcus aureus (MRSA) has surpassed HIV as the most deadly pathogen in the United States, and accounts for over 100,000 deaths per year worldwide. In Orthopaedics there is great concern over the very poor outcomes of two-stage revision total joint replacement (TJR) surgery from MRSA osteomyelitis (OM), which has a 30-50% failure rate. Thus, non-traditional interventions like passive immunization are warranted, particularly for immunocompromised patients and the elderly who are typically poor responders to active vaccines. Previously, we identified the S. aureus peptidoglycan hydrolase glucosaminidase (Gmd) as a protective antigen in a murine model of implant-associated OM. Since this enzyme is essential for cell wall digestion during bacterial growth, and no significant genetic variation has been identified among all clinical strains, we hypothesized that effective anti-Gmd antibodies would have multiple mechanisms of action, including promotion of opsonophagocytosis and direct inhibition of enzyme function. Thus, the aim of this study was to evaluate the activity of a high affinity IgG1 anti-Gmd monoclonal antibody (1C11) in vitro and in vivo.

METHODS: Antibodies: 1C11 was produced from a hybridoma cell line grown in DME media containing 10% FBS, purified from the culture supernatant using Protein G sepharose, concentrated, dialyzed, and sterile-filtered. IP Western blotting: 1 ml of supernatants from overnight LB cultures of MRSA strains USA100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, and 1100 were immunoprecipitated with 1C11-Protein G Dynabeads and immunoblotted with 1C11. 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 control group was treated with PBS only. Samples were then plated onto silicon chips, fixed, dehydrated, and coated with gold for visualization by SEM. In Vivo Challenge: Five-week-old female BALB/cJ mice received an intraperitoneal injection of PBS or 1 mg of purified anti-Gmd mAb 1C11 3 days prior to surgical transilibration of a steel pin coated with 500,000 CFU of a biofilmogenic strain of S. aureus, Xen29. Bioluminescent imaging (BLI) was performed on days 0, 3, 5, 7, 10, and 14. Micro-CT: Mice were sacrificed at day 14 and tibiae were analyzed by high-resolution (10.5 μm) micro-CT. The pins were analyzed by SEM to assess in vivo biofilm formation. All protocols involving the use of animals were approved by the university’s Animal Care and Use Committee, and followed all state and federal guidelines.

RESULTS: IP-Western blotting revealed that 1C11 specifically binds to the 56kDa Gmd expressed by all known genetically distinct MRSA strains (data not shown). In vitro SEM revealed that S. aureus treated with 1C11 did not undergo complete binary fission, and grew as large clusters that fell out of suspension. In contrast, S. aureus-only controls grew mostly as single-cell suspensions (Fig. 1). Quantification of random fields demonstrated a significant (p<0.05) effect of 1C11 on cluster size vs. the control (data not shown).

In vivo evaluation of 1C11 effects in our murine model demonstrated that passive immunization significantly protects ~50% of the animals from implant-associated OM. BLI analysis revealed a clear difference between the protected mice, which displayed markedly lower BLI levels vs. placebo at all time points (Fig. 2A). Cross-sectional BLI analysis at the peak of infection (Day 3) revealed a biphasic response, in which half of the 1C11 treated mice were “protected” and had values below the threshold of the placebo group (Fig. 2B). Consistently, micro-CT demonstrated that the sequestrum found in the tibiae of infected mice (Fig. 3A) was completely absent in the animals protected by 1C11 (Fig. 3B). Furthermore, SEM confirmed the presence of biofilm that covered the entire surface of the explanted pins from the infected mice (Fig. 3C), which was greatly reduced in the protected 1C11 treated mice (Fig. 3D).

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