Synergistic effects of combination antibiotic and immunotherapy demonstrated in a novel murine one-stage exchange model of methicillin-resistant Staphylococcus aureus implant-associated osteomyelitis

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INTRODUCTION: Prosthetic joint infection (PJI) is one of the most serious complications after total joint arthroplasty, and its incidence continues to rise.1 Unfortunately standard PJI treatments including prolonged antibiotic therapy are ineffective for many patients who progress to severe disability, amputation and death. Staphylococcus aureus (S. aureus) is the most common pathogen in PJI, approximately 50% of which is methicillin-resistant S. aureus (MRSA) and reinfection rates from MRSA PJI are reported to be 15-40%.2,3,4 Thus, there is a great need for novel adjuvant therapies. To this end, we developed a novel passive immunotherapy using an anti-glucosaminidase (Gmd) monoclonal antibody (1C11), and demonstrated its efficacy in a prophylactic model.5 However, as our goal is an adjuvant immunotherapy for PJI, we aimed to: 1) develop a novel one-stage exchange murine femoral plate model of MRSA implant-associated osteomyelitis; and 2) utilize this model to demonstrate the synergistic effects of combination vancomycin (VCM) and anti-Gmd therapy.

METHODS: All experiments were performed on IACUC approved protocols. Surgical procedures: The 1-stage exchange model was designed by modifying a plated femoral osteotomy model.7 As a primary surgery, a 4-hole titanium plate was implanted into the femur of 10-week-old female BALB/c mice with 2 titanium screws inserted into the innermost screw holes. The distal screw was incubated in an overnight culture with a bioluminescent MRSA strain (USA300LAC::lux) for 20 minutes prior to insertion (n=20), or directly inserted as negative control (n=5). All implants were removed 7 days after primary surgery, followed by thorough irrigation with sterile PBS and debridement. Afterwards, the femur was fixed again with a new sterile plate and 2 screws inserted into the outermost unused screw holes, and the mice were euthanized on day 7 post-revision. Treatment Groups: 5 cohorts of mice (n=5) were studied: Group 1) sterile implant control; Group 2) infected placebo (40mg/kg irrelevant Ig control injected i.p. on day 6 post-infection); Group 3) anti-Gmd monotherapy (40mg/kg 1C11 IgG2a injected i.p. on day 6 post-infection); Group 4) VCM monotherapy (110 mg/kg once daily subcutaneously from day 7 until sacrifice); and Group 5) combination (Combo) anti-Gmd and VCM therapy. Outcomes: All outcomes were performed as previously described and included: longitudinal bioluminescent imaging (BLI) performed on days 0, 1, 3, 5, 7, 8, 10, 12, and 14; x-rays on days 0, 3, 7, 10, and 14; day 14 serology; colony forming unit (CFU) on explanted hardware; ex vivo micro-CT; and histology.

RESULTS: Radiographic evidence of extensive osteolysis adjacent to the contaminated screw, and at the end of the plates, was apparent on day 14 x-rays from all mice in the placebo and ant-Gmd monotherapy Groups (Fig. 1A). This osteolysis appeared to be reduced by VCM, remarkably decreased by Combo, and was completely absent in the sterile implant Group. All infected mice displayed the documented pattern of BLI over the first week,5,7 in which BLI peaked due to planktonic growth up to day 3, and declined during the biofilm stage (Fig. 1B). This confirmed that all experimental mice had similar infections prior to treatment. While this same BLI pattern repeated following revision surgery in the placebo Group, the anti-Gmd and VCM Groups displayed trends of 1.8 and 3.6-fold reduction in peak BLI on day 10, respectively. However, only the Combo Group displayed a significant 7.2-fold reduction in BLI on day 10 (p<0.05 vs. placebo), and achieved the sterile implant BLI level by day 14. While anti-Gmd monotherapy had no effect on CFU (Fig. 1C), both VCM and Combo Groups had significantly reduced total CFU counts on all implants (p<0.05 vs. placebo). Synergistic effects were also apparent from the finding that only the Combo therapy had an infected mouse with undetectable CFU on every implant.

DISCUSSION: A major obstacle towards the development of an adjuvant immunotherapy for PJI is the absence of a small animal model, as all vaccine work to date has been done is prophylactic models. To address this, here we describe a novel one-stage exchange murine model of MRSA infected implants with quantitative outcomes. Additionally, we utilized this model to generate the first in vivo evidence of synergistic effects of antibiotic and immunotherapy, as evidenced by the significant efficacy that was only seen in the Combo Group. In terms of a potential mechanism for the synergy, a recent study reported that the amidase subunit of S. aureus autolysin is a target of VCM.7 Thus, anti-Gmd immunotherapy, which targets the other subunit of autolysin could have synergistic effects with VCM by completely neutralizing this critical enzyme. However, future studies are necessary to validate the synergistic effect.

CLINICAL SIGNIFICANCE: New therapies for PJI are need to treat patients with life threatening MRSA infections. To this end, here we describe a novel 1-stage revision murine model with quantitative outcomes, and preclinical evidence that adjuvant immunotherapy is feasible and synergistic with revision surgery and VCM therapy. These results support the further development of anti-Gmd immunotherapy for the prevention and treatment of MRSA osteomyelitis.


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Figure 1. Efficacy of VCM and anti-Gmd mono and combination therapy in the 1-stage revision model. A) Representative day 14 radiographs of the femoral plate prior to explant illustrate the level of osteolysis (yellow arrowheads). B) Longitudinal BLI presented as the mean ± SD. C) CFU on the explanted screws and plate. Note that all sterile implants had no CFU, and all placebo and anti-Gmd monotherapy implants had >10^5 CFU. In the VCM Group 1 proximal screw and 2 distal screws, in the Combo Group 1 plate, 2 proximal screws, and 2 distal screws had no CFU.