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O0247 The MEDI4893 monoclonal antibody epitope on S. aureus alpha toxin is highly conserved and critical for toxin function

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Background: Alpha toxin (AT) is a cytolytic pore-forming toxin and pathogenic determinant for many *Staphylococcus aureus* diseases. We previously generated MEDI4893, a human AT neutralizing mAb, currently in phase 2 clinical testing. MEDI4893 potently neutralizes the toxin in vitro and in vivo by blocking AT binding to target cells. MEDI4893 epitope is conformational and discontinuous, comprising amino acid residues 177-200 and 261-271 on AT. This epitope was found to be highly conserved in studies surveying AT protein sequences from >1500 clinical isolates suggesting that the amino acids within the epitope are critically important for AT function. To test this hypothesis and gain further understanding into the effect of mutations in MEDI4893 epitope on mAb binding and neutralization, we employed site directed mutagenesis on the nine MEDI4893 contact residues in AT.

Materials/methods: Nine mutants were generated by alanine scanning mutagenesis, cloned, expressed and purified from E. coli. These mutants were were evaluated for functional activity and neutralization by MEDI4893 in cytolytic in vitro assays and in a mouse dermonecrosis model. Affinity to MEDI4893 was measured by Biacore and binding to a human epithelial cell line was assessed by flow cytometry.

Results: 8/9 mutants exhibited >2-fold loss in lytic activity on rabbit red blood cells and a lung epithelial cell line resulting from a defect in cell binding as determined by flow cytometry. MEDI4893 binding affinity was reduced >2-fold in 7/9 mutants. The lowest measurable mAb affinity constant (K_D) of 3.81 nM in mutant K266A while no binding was detected to mutant W187A. However, in all mutants with detectable lytic activity, MEDI4893 effectively neutralized their activity in vitro and in vivo. Additionally, when the mutations were introduced into the AT gene of a *S. aureus* clinical isolate, the mutant expressing strains exhibited less severe lesions in a dermonecrosis infection model consistent with their lytic activity in vitro and were effectively neutralized by MEDI4893.

Conclusions: Site directed mutagenesis in MEDI4893 epitope largely compromised AT activity, but MEDI4893 retained binding and neutralization activity for all partially functional AT mutants, supporting a critical role for this toxin domain and low potential for antibody resistance.