Mapping of neutralizing monoclonal antibody binding epitopes on Clostridioides difficile toxin proteins

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Clostridioides difficile is a spore-forming Gram-positive bacterium that can infect exposed subjects with weakened immune systems while undergoing antibiotic therapy. A change in the normal gut microbiome and antibiotic resistance lead to C. difficile infection (CDI). CDI is a leading cause of hospital acquired diarrhea and pseudomembranous colitis. Infection is mediated by large proteins, toxins A and B, that share distinct functional domains. Genetic and chemical modification of these toxins eliminate cytotoxicity while preserving critical epitopes for the generation of neutralizing antibodies induced through immunization.

Here we describe the identification of binding epitopes for a subset of antibodies elicited in response to immunization with toxin antigens using a combination of hydrogen-deuterium exchange mass spectrometry (HDX-MS) and electron microscopy (negative staining and cryogenic EM). The synergistic use of these techniques enabled the mapping of binding epitopes for monoclonal antibodies (mAbs) specific to distinct functional domains for each toxin.

To visualize a direct toxin-antibody interaction, cryo-EM was used to structurally characterize a preserved toxin epitope. Here we report a structure at 3.25 Å resolution of full-length toxoid A in a complex with the Fab fragment of a mAb that is able to neutralize toxin cytotoxicity. This provides structural insight into the mechanism of toxin A neutralization by this mAb and serves as a benchmark for investigating the binding epitopes of other toxin neutralizing mAbs by cryo-EM.