Title:

A VHH-based Neutralizing Agent (VNA) Targeting Both TcdA and TcdB Toxins Protects Mice and Gnotobiotic Piglets from the Pathology of Clostridium Difficile Infection

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Abstract:

Clostridium difficile infection (CDI) is one of the leading causes of nosocomial infection worldwide and is becoming an increasing problem as a community acquired infection as well. Symptoms of CDI range from mild diarrhea, pseudomembranous colitis, toxic megacolon, to organ failure and death. The emergence of antibiotic resistant strains and the increase in relapse or recurrence have complicated treatment of the disease and increased hospital stays, morbidity, and mortality among patients. Therefore it is critical to develop new therapeutic treatments for this disease.

We immunized alpacas with atoxic mutant toxin A or B antigens (the two major virulence factors of CDI). B cells were isolated from the immune alpaca and phage display libraries were generated displaying heavy-chain-only Ab VH (VHH) domains. The libraries were panned against native TcdA or TcdB toxins yielding about 20 unique, toxin-binding VHHs. VHHs were identified that recognize each of the major toxin domains on TcdA and TcdB, including the glucosyltransferase, transmembrane and receptor binding domains. These VHHs were tested in a cytotoxicity neutralization assay and approximately half neutralized toxin activity in vitro. Several VHH heterodimers were produced in which two neutralizing VHHs were expressed as a single protein separated by a flexible spacer peptide. These heteromultimeric VHH-based neutralizing agents (called VNAs) were generally found to have substantially enhanced toxin neutralization potency in cell assays compared to a pool of the component monomer VHHs. To create a single VNA capable of neutralizing both TcdA and TcdB, a VHH heterotetramer (ABBA) was produced containing the two most potent neutralizing VHHs to each toxin. ABBA was expressed and purified and shown to neutralize both TcdA and TcdB with high potency. ABBA has now been tested as a therapeutic agent in animals and demonstrated excellent efficacy in preventing disease symptoms in both mouse and gnotobiotic piglet models of CDI.