Successful development of recombinant human diphtheria antitoxin: a project update

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Introduction

Diphtheria is a disease caused by toxigenic strains of diphtheria toxin-producing *Corynebacterium spp.* Vaccination prevents clinical development of the disease which is consequently rare in regions where immunization campaigns are in place. Regions with limited vaccine coverage nevertheless experience outbreaks that must be treated with diphtheria antitoxin (DAT), an equine serum product that carries the risk of causing adverse reactions including serum sickness or transmission of undetected infectious agents between species. Equine DAT is difficult to stockpile with frequent supply chain and distribution problems in addition to short shelf life.

At the 10th World Congress, we announced our project to develop human monoclonal antibodies that neutralize DT while avoiding the cross-species and shelf life problems associated with equine blood products. Here we report the successful development of those antibodies and our next steps toward the long term goal of replacing equine DAT with a recombinant antibody product of consistent identity that can be produced in cell culture.[1]

Methods and results

Antibody phage display is a validated approach that facilitates selection of sequence-defined DT-binding antibodies that can be manufactured under reproducible conditions in cell culture directly from human antibody gene libraries *in vitro.* Using two naïve human antibody libraries (HAL9 and HAL10) and two immune libraries created for this project (VJN and CD138+), 400 DT-binding antibodies were selected. Of this pool, Vero cell toxin neutralization tests (TNT) yielded 34 DT-neutralizers in IgG format.

Three of these antibodies were selected for additional Vero cell TNT testing, each targeting one of the three DT structural domains (catalytic (C), transmembrane (T), and receptor binding (R) domains), against increasing toxin concentrations. Individual antibodies lost neutralization capacity at higher DT doses, yet showed strong neutralizing activity when the antibodies were used in combinations of two or three.

Subsequently, the neutralization efficacy of these antibodies was assessed using a non-lethal guinea pig intradermal challenge assay based on the method described in the European Pharmacopoeia, confirming that these three antibodies in combination, both in pairs and triples, resulted in a clinically relevant neutralization potency (79 IU/mg, Table 1).

Table 1: Non-lethal *in vivo* neutralization at Lr/100 toxin dose level* for antibodies and antibody combinations

Antibody clone(s)	[IU/mg]
ewe375-D4 (anti R-domain)	<0.72
ewe375-H4 (anti C-domain)	<6.4
ewe372-F6 (anti T-domain)	<1.23
ewe375-D4 + ewe375-H4	79.4
ewe375-D4 + ewe372-F6	79.4
ewe375-H4 + ewe372-F6	79.4
ewe375-D4 + ewe375-H4 + ewe372-F6	79.4

*Lr/100 is the smallest amount of DT that causes a weak but detectable erythema in the presence of 0.01 IU of reference DAT antitoxin.

Discussion

Our results suggest that the neutralizing potency of these three antibodies in combination is comparable and likely superior to the results obtained for single monoclonal antibodies developed elsewhere. The use of antibody combinations that bind more than one domain on the target toxin may represent a robust therapeutic approach and are being explored as candidates for further regulatory and clinical development as a replacement for equine DAT.

Antibody phage display is an efficient and affordable technology that can be applied to the development of antibodies for use in therapeutic and diagnostic contexts. Based on the success of this project, the PETA International Science Consortium is funding similar research to develop recombinant human antibodies that neutralize black widow spider venom as a replacement for equine antivenom.

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[1]Wenzel EV, Bosnak M, Tierney R, Schubert M, Brown J, Dübel S, Efstratiou A, Sesardic D, Stickings P, Hust M. Human antibodies neutralizing diphtheria toxin in vitro and in vivo. Sci Rep. 2020; 10(571): doi:10.1038/s41598-019-57103-5