Development of recombinant human anti-diphtheria toxin neutralizing antibody for diphtheria therapy

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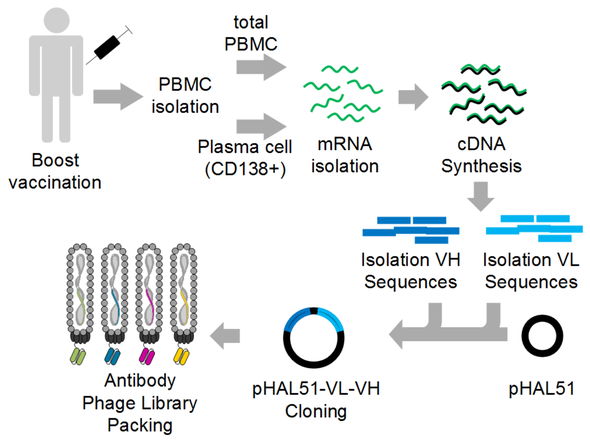
Abstract

Diphtheria is a disease caused by toxigenic strains of *Corynebacterium spp.* that produces diphtheria toxin (DT). The disease is well controlled by immunization and is therefore rare in countries with sufficient immunization coverage. However, diphtheria represents a significant health problem in countries with poor immunization coverage or disrupted immunization programs. Therefore, there is a need to maintain a stockpile of therapeutic diphtheria antitoxin (DAT) – even in countries where the disease is well controlled. Currently, diphtheria is still treated with equine sera in the same way it was treated more than 100 years ago by Emil von Behring. Besides, DAT is scarcely supplied and frequently unavailable to patients.

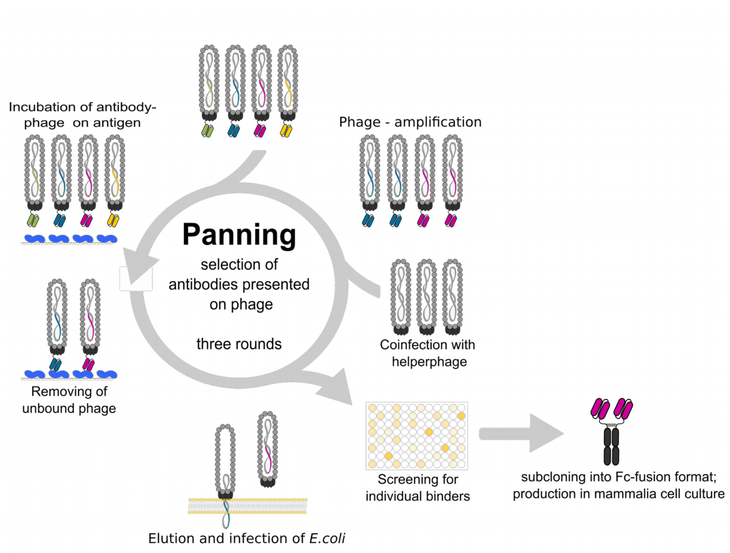
The aim of the project is to develop human monoclonal antibodies against DT. The long term goal is the replacement of equine DAT sera with a recombinant antibody product produced in cell culture.

Human antibody gene libraries



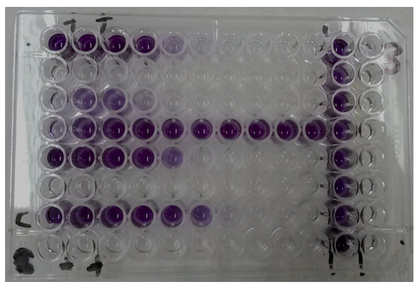
Using the naive human antibody libraries HAL9 and HAL10, and 2 immune libraries (VJN and CD138+), 171 monoclonal scFv antibodies against diphtheria toxin were generated.

Antibody phage display



Vero cell toxin neutralisation test (TNT)

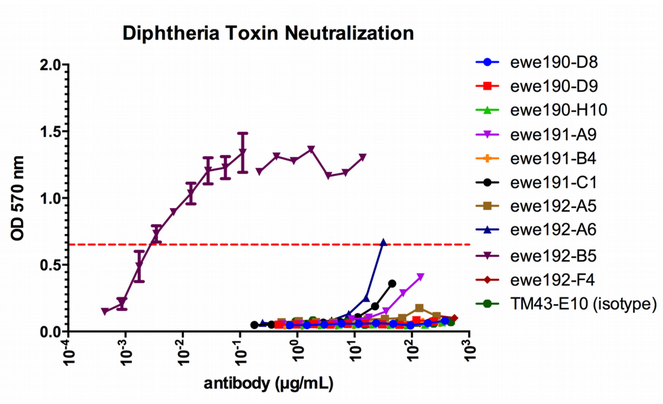
Chosen scFv-Fc antibodies were tested for *in vitro* neutralization potential.
Toxin and antibodies were co-incubated in media (60 min at RT). Vero Cells were incubated with the antibody- toxin mixture for 6 days. The mitochondrial activity was determined by a MTT assay.

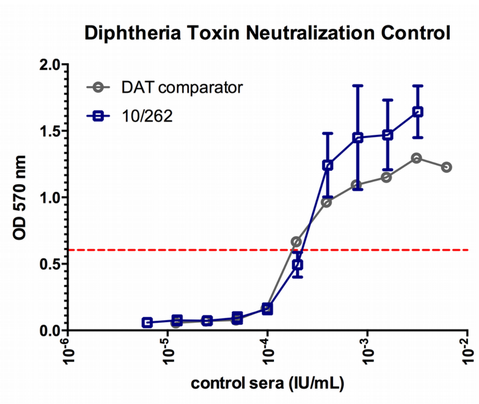


Results:

A total of 276 scFv antibodies were produced in *E. coli* cells and screened for recognition of diphtheria toxin by ELISA. 171 DT specific scFv were identified, thereof 127 antibodies are unique. 105 of these antibodies have a lambda and 22 antibodies have a kappa light chain. Randomly 12 antibodies, 4 out of each library, were cloned into scFv-Fc format and expressed by transient transfection in HEK293-6E cells. 10 scFv-Fc were producible. Antibodies were screened for recognition of diphtheria toxin, diphtheria toxoid and CRM197 by ELISA. All antibodies recognized the diphtheria toxin, whereas the diphtheria toxoid was only recognized by antibodies from immune libraries. Just four of the antibodies showed a similar binding to diphtheria toxin than to the non-toxic mutant of diphtheria toxin called CRM197. The 10 unique scFv-Fc antibodies were tested for the ability to inhibit cytotoxicity of diphtheria toxin using a cell-based neutralization assay with Vero cells (TNT assay). 2 scFv-Fc demonstrated significant toxin neutralization activity. The best neutralizing antibody (ewe192-B5) has a half-maximal effective neutralization concentration (EC50) of 0.024 nM.

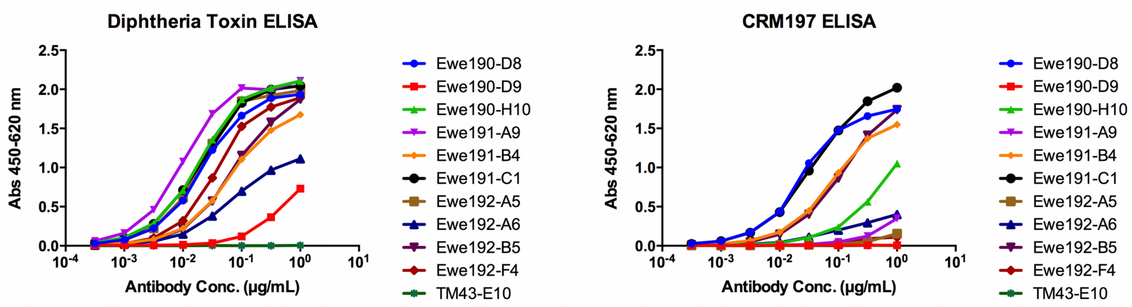
Vero cell TNT

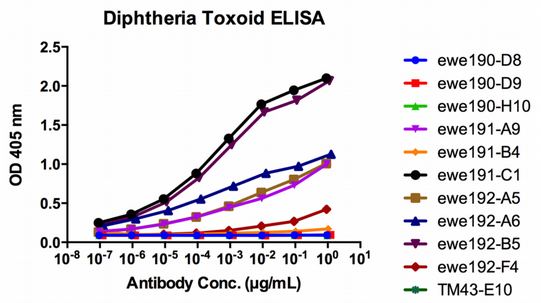




Preliminary results for *in vitro* neutralization combined from four assay plates.

Antibody binding (ELISA)





Conclusion and outlook:

Diphtheria toxin specific scFv antibodies are selectable through phage display. Next, all the selected antibodies will be characterized regarding neutralization activity, stability and furthermore their producibility in IgG format. Due to the human genetic origin of the generated antibodies, they are potential lead candidates for future clinical development.

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