

## Potential of the *in vivo* neutralization capacity of anti-BoNT antibodies through a novel erythrocyte immunoadherence mechanism.

S. P. Adekar<sup>1,2</sup>, A. T. Segan<sup>1</sup>, R. Bermudez<sup>2</sup>, C. Chen<sup>1</sup>, B. Kapadnis<sup>3</sup>, L. L. Simpson<sup>4</sup>, P. M. Simon<sup>5</sup>, S. K. Dessain<sup>1,2</sup>

<sup>1</sup>Lankenau Institute for Medical Research, Wynnewood, PA, <sup>2</sup>Immunome Inc, Wynnewood, PA, <sup>3</sup>Dept. of Microbiology, University of Pune, Pune, India, <sup>4</sup>Thomas Jefferson University, Philadelphia, PA, <sup>5</sup>Augmenta Biologicals, LLC, Wynnewood, PA.

**Background:** An essential factor in neutralization of the botulinum neurotoxin (BoNT) is clearance of the toxin from the blood circulation. Polyclonal BoNT-specific antibodies form immune complexes with BoNT, which are rapidly re-distributed from the blood circulation into the spleen and liver, where they are presumably captured by reticuloendothelial (RE) cells through Fc receptor and/or complement-mediated mechanisms. We have used a novel immunoadherence mechanism to enhance the ability of BoNT-specific human monoclonal antibodies to augment clearance of BoNT from the circulation and thereby increase their *in vivo* neutralization capacity.

**Methods:** We employed a novel fusion protein (FP), which consists of a streptavidin moiety (StAv) in frame with an antibody domain specific for the red blood cell (RBC) glycoprotein A surface protein (scFv). We have used this fusion protein, along with biotinylated antibodies, to create “augmented antibody complexes”, specific for BoNT, which can link toxin to the RBC surface. Complexes were formed by incubating the FP with biotinylated human monoclonal antibodies and BoNT *in vitro* and then injected into the tail veins of 25 gram Swiss Webster mice. Mice were observed for signs of BoNT intoxication and for survival. In pre- and post-exposure models of BoNT intoxication, we administered toxin intraperitoneally and augmented antibody complexes intravenously. We also evaluated the ability of the complexes to adhere to erythrocytes *in vitro* by flow cytometry.

**Results:** Incorporation of BoNT-specific human monoclonal antibodies into augmented antibody complexes that included the FP increased the neutralization of the antibodies. Single antibodies able to completely neutralize a lethal dose of toxin (6A and 14LCA) could neutralize increased doses of BoNT/A1, while an antibody with only partial neutralizing activity (13A) was able to fully neutralize a lethal BoNT/A1 dose. An augmented antibody complex that included neutralizing antibodies 6A and 14LCA could neutralize 5000 LD<sub>50</sub> BoNT/A. In the post-exposure model of intoxication, augmented antibody complexes gave complete protection from a lethal BoNT/A1 dose (100%) when administered within 2 hours of toxin exposure, and 87.5% and 62.5% protection when they were administered after 3hrs and 4hrs respectively. In the pre-exposure prophylaxis model, mice were fully protected for 72 hours following administration of an augmented antibody complex. Activity *in vivo* correlated with the ability of the augmented antibody complexes to bind erythrocytes *in vitro*

**Conclusions:** We have used a novel fusion protein, FP, to create augmented antibody complexes that can dramatically potentiate the *in vivo* neutralization capacity of anti-BoNT antibodies through a novel erythrocyte immunoadherence mechanism. Antibody complexes provide potent activity in post-exposure intoxication models and stable protection in pre-exposure models.