

A novel immunoadherence mechanism for the clearance of botulinum neurotoxin using a RBC-targeting fusion protein

Sharad P. Adekar^{1,2}, Andrew T. Segan¹, Rodney Bermudez², Cindy C. Chen¹, B. P. Kapadnis³, Lance L. Simpson⁴, Paul M. Simon⁵, Scott K. Dessain^{1,2}

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

Background: Botulinum neurotoxin (BoNT) is a Category A Select Bioterror agent because of its extreme lethality and ease of production. Immunotherapy is presently considered to be the most effective immediate response to BoNT exposure. The most important factor in BoNT neutralization is clearance of the toxin from the blood circulation. Polyclonal BoNT-specific antibodies form immune complexes with BoNT that 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) (from Augmenta Biologicals, LLC, Wynnewood, PA), which consists of a streptavidin moiety (StAv) in frame with an antibody domain specific for the red blood cell (RBC) glycoporphin A surface protein (scFv). We have used this fusion protein along with biotinylated antibodies (referred to as “augmented antibodies”) against botulinum toxin to adhere botulinum toxin to the RBC surface. We incubated the FP with biotinylated human monoclonal antibodies and BoNT prior to injection into the tail veins of 25 gram Swiss Webster mice. Mice were observed for signs of BoNT intoxication and for survival. For a more realistic post-exposure model of BoNT intoxication we also administered toxin i.p. followed by administration of augmented antibodies i.v. We also evaluated the ability of the complexes to adhere to erythrocytes in vitro.

Results: We have used ultra-high affinity human monoclonal antibodies which we have cloned using our novel hybridoma method. We tested whether a non-neutralizing antibody could become neutralizing and, whether non-neutralizing doses of antibodies could become neutralizing if the biotinylated antibodies were coupled to FP. The 13A antibody which was non-neutralizing protected mice at 10 LD50 when administered at 1.5ug along with FP. The 6A antibody was not protective at 2.5 LD50 when administered at 1.5 µg, whereas 1.5 µg 6A plus FP was neutralizing at 10 LD50. The maximum dose of BoNT/A neutralized by 100 µg 4LCA was 25 LD50, whereas 1.5 µg 4LCA bound to FP could neutralize up to 250 LD50. Combination of 6A with 4LCA along with FP could neutralize up to 5000 LD50 when used at only 3 µg of each antibody.

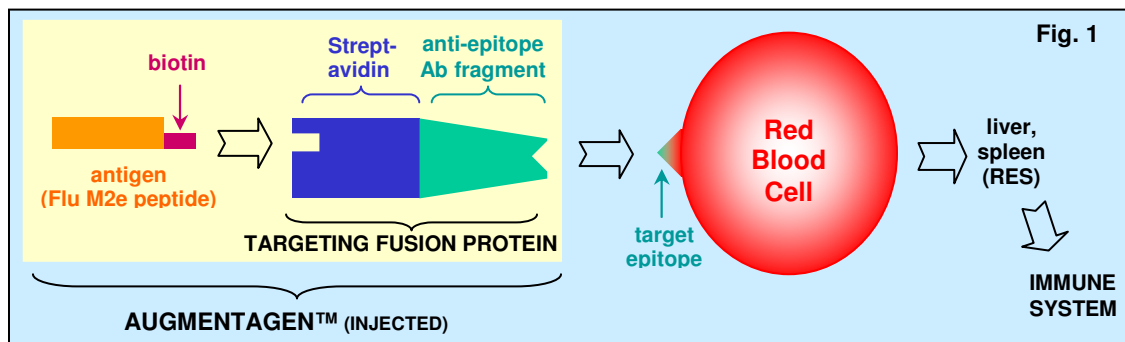
To simulate a real-world scenario, we created a post-exposure model of toxin where BoNT/A was administered first and antitoxin antibody coupled with FP combinations were given at different time intervals afterwards. An augmented antibody combination gave complete protection (100%) when administered 2hrs after toxin, 87.5% and 62.5% protection when they were administered after 3hrs and 4hrs respectively. We have also characterized the in vitro binding of the FP-antibody-toxin complex to RBC's.

Conclusions: We have used a novel fusion protein, FP, which can dramatically potentiate the in vivo neutralization capacity of anti-BoNT antibodies through a novel erythrocyte immunoadherence mechanism.

ENHANCED IMMUNIZATION VIA RED BLOOD CELLS

Augmenta Biologicals is a seed stage company developing an invention for augmenting the potency of immunizations. This enabling technology platform is useful for many antigens, even those for which it is difficult to elicit a strong immune response by conventional means.

Technology. The strategy relies on a natural function of red blood cells, which, in addition to providing oxygen to cells and tissues, also collect foreign substances in the blood for clearance by the liver and spleen, a process that is accompanied by a very efficient presentation of these substances to the immune system. We have devised a molecule that targets immunogens to red blood cells to take advantage of this route. The molecule consists of two parts: one part is an antibody fragment that homes the molecule to the red blood cell surface in vivo; the other part, streptavidin, binds to the immunogen, requiring only a simple chemical reaction, biotinylation



(Fig. 1).

In initial tests in mice using a flu virus peptide known to be a weak antigen, a strong immune response was elicited, more than **2,000-fold greater** when the virus protein was coupled to our targeting fusion protein

(FP) than by itself, or if combined with alum (Fig. 2). This result validates the strategy and paves the way for its evaluation as an adjunct to veterinary and human vaccines. The approach can elicit higher immune responses and allow smaller doses thus reducing manufacturing quantity and timing, important attributes in broad immunization campaigns. The same technology also helps clear toxins of biodefense relevance from the blood, increasing the potency of antitoxin antibodies by **more than 600-fold** (Fig. 3). The technology can also be readily adapted to other animal species for production of monoclonal and polyclonal antibodies.

Intellectual property. US and international patent applications (WO 2007150020) have been filed and are owned by the Company.

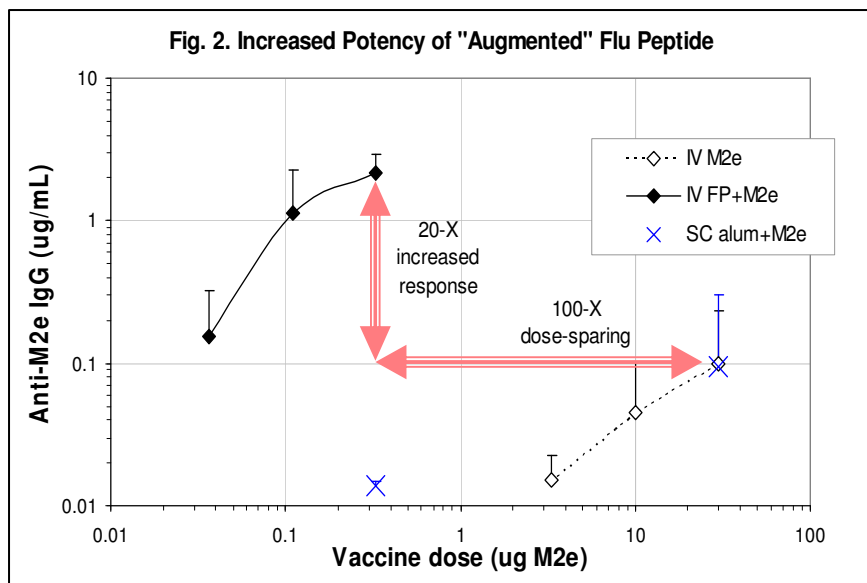
Team. Paul M. Simon, PhD, is the founder, President and CSO of Augmenta and inventor of the technology. He is an independent entrepreneur with over 25 years of experience in the management of biopharmaceutical discovery R&D. **Advisors.** Bernard Rudnick, finance & strategy (investment advisor, Cap-Genic assoc.). Ron Saldarini, PhD, human vaccines (former President Wyeth Vaccines). Joy Cavagnaro, PhD, regulatory affairs. Elizabeth Song, PhD, business development. Scott Dessain, MD, PhD, biodefense Lankenau Inst. Med. Res.), Michael O'Hara, PhD, animal health (BARDA/HHS, ex-Pfizer). William Wong, PhD, entrepreneurial strategy, James M. Pachence, PhD, Corporate Development, (serial entrepreneur).

Business approach. Revenue creation can be accomplished via multiple business tracks: (1) custom antibody production in mice (available immediately) and other species, an unregulated opportunity; (2) induction of immunity (vaccination) offering dose-sparing and avoidance of immune adjuvants in humans, a \$22B market growing at 10% annual, and livestock and companion animals, a \$4B market growing at a rate of 12%; and (3) clearance of toxins from the circulation, of interest to biodefense and other applications. Companies with immune enhancing technologies have been acquired in recent years (Corixa by GSK in 2005, \$325M and Coley by Pfizer in 2007, \$600M).

Augmenta Biologicals is exploring investment, licensing, corporate partnership and collaboration opportunities.

Augmented Immunity

Augmenta's immunotargeting strategy uses a fusion protein (FP) comprised of a fragment of an anti-red blood cell monoclonal antibody and streptavidin, a molecule that binds biotin very tightly. The test vaccine immunogen here is M2e, a peptide found on the surface of all strain A influenza viruses, including H5N1, the pandemic "bird flu" strain, H1N1, or "swine flu" or seasonal flu. The M2e peptide bearing a biotin group was combined with the FP ex vivo and injected into mice using a conventional immunization schedule. Serum was analyzed for the production of anti-M2e antibodies. Mice immunized with M2e peptide alone or peptide in combination with the adjuvant alum generated a modest IgG response. By comparison, much smaller amounts of M2e coupled to the FP ex vivo generated a potent immune response (Fig. 2). In fact, 37 ng of FP-borne M2e peptide elicited a more potent immune response than 30 μ g of unconjugated peptide. **The FP imparted a total immunogenicity increase of more than 2,000-fold over that of peptide alone or with alum adjuvant.**



Enhanced Toxin Clearance

An independent evaluation of the fusion protein (FP) was recently conducted in the lab of Dr. Scott Dessain at the Lankenau Institute for Medical Research in Wynnewood, PA. He did not use the FP for immunizing mice, but rather to help clear botulinum neurotoxin from the bloodstream. He has high affinity human monoclonal antibodies (HuMabs, A and C in Fig. 3) against the toxin which neutralize a limited dose of toxin. The current hypothesis is that when antibodies are injected with botulinum toxin, toxin-Mab immune complexes form and are present in the blood for some time. Binding by HuMabs prevents toxicity, however; unless rapidly removed from the circulation, some toxin can detach from the antibody and damage neurons, causing lethal paralysis. In recent experiments, these HuMabs were biotinylated and coupled to Augmenta's FP which led to the complete healthy **survival of mice against up to 5,000 times the lethal toxin dose** (Fig. 3). The interpretation is that the fusion protein transports the Mab-toxin complex to the RBC and thence to the liver and spleen where it is rapidly degraded, preventing toxin from leaching from the antibody complex. This provides added support to the proposed mechanism of action of the FP in a non-immunization setting and demonstrates another application for this technology.

