Development of a Recombinant Antibody-Based Treatment of Snakebites

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Improving Antivenom to Save Lives and Limbs

Antivenom for snakebites is produced by immunization of large mammals with snake venom using a traditional and expensive method developed in the 1890’s. Due to the animal origin, the products are highly immunogenic and come with a high risk of adverse side effects such as serum sickness and anaphylaxis, possibly leading to death [1].

This project aims at replacing existing snake antivenoms with a mixture of recombinant, humanized antibodies produced by modern cell-based fermentation technology [2]. It is anticipated that such an antivenom will reduce the current high risk of severe side effects, reduce cost, and thereby can be sold at 1/10 of the current price making the essential medicine available for > 700 M Africans [4].

Modern day technology allows development of monoclonal antibodies (mAbs) targeting snake toxins, however, identification, characterization of immunogenic features (B-cell epitopes), and availability of purified snake toxins or non-toxic analogs currently constitute major bottlenecks blocking the development of recombinant mAbs. We have set out to remove these bottlenecks starting by mapping antibody binding sites of existing horse-derived products and purified antibodies from snakebite victims using high-density peptide microarrays. Moreover, we are developing homology models of all relevant mamba toxins to map conserved sites and identify key residues for toxicity.

Modeling Short Neurotoxin 1 from Mamba Snakes

Figure 3 – Homology model of clinically relevant toxin from mamba (Dendroaspis) snakes. Surface and cartoon representations of short neurotoxin 1 (SN1) illustrating interspecies variation and the idea of finding one cross-reactive mAb. SN1 is a member of the large and diverse family of three-finger toxins (3FTx). SN1 is known to antagonize the neuromuscular nicotinic acetylcholine receptor (nAChR) using finger 1 and 2 for binding [5]. Templates for homology model: IER5 (crystal structure of mutant related Erabutoxin a from a sea snake with low affinity for nAChR) [7] and 2QC1 (nAChR bound distantly related α-bungarotoxin from the many-banded krait) [8].

Figure 4 – Identification of residues important for toxicity using homology modeling. Left: Key interactions between nAChR and finger 2 of the co-crystallized α-bungarotoxin (orange). RS6 highlighted (yellow). Right: Homology model of mamba SN1 aligned to nAChR reveals an arginine residue, R31 (yellow), at a similar position in binding pocket. Below: Alignment of the three mamba SN1’s and α-bungarotoxin.

Challenges in the near future

Figure 5 – Schematic overview of upcoming challenges related to protein research.

References

[5] Erabutoxin a from a sea snake with low affinity for nAChR [7] and 2QC1 (nAChR bound distantly related α-bungarotoxin from the many-banded krait) [8].

Venue analysis


Antibody identification

Screening of phage display libraries

Expression and purifying toxins and non-toxic analogs

Validation of correct protein fold

Validation of physiological activity

Sequenceing of binding antibodies

Validation in vitro and in vivo

Modeling of protein-protein interactions

Strong IgG response against the nAChR antagonist α-cobratoxin found in serum from human donor (unpublished results)

Investigate other toxin targets of serum antibodies

Isolation of antibodies based on toxin targets

Sequencing of binding antibodies

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Figure 1 – The Sub-Saharan antivenom crisis. Overview of the vicious cycle fueled by the current production methods of antivenom and dangerously inappropriate products of Indian origin marketed by unscrupulous manufacturers dominating the unregulated African market [4]. WHO describes snakebites as one of the Worlds most neglected tropical diseases and the antivenom situation in Sub-Saharan Africa as a long standing crisis [5].

Figure 2 – Schematic overview of research approach.

Figure 5 – Schematic overview of upcoming challenges related to protein research.