High-throughput epitope profiling of snake venom toxins
unveiling the complexity of antigen-antibody interactions of antivenoms

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High-throughput epitope profiling of snake venom toxins – unveiling the complexity of antigen-antibody interactions of antivenoms

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Introduction
Insight into the molecular details of polyclonal antivenom antibody specificity is a prerequisite for accurate prediction of cross-reactivity and can provide a basis for design of novel antivenoms. In this work, a high-throughput approach was applied to characterize linear epitopes in venom toxins from four African mamba and three neurotoxic cobra snakes obtained from public databases.

Studying linear epitopes using peptide microarrays
Generation of an in silico library of 12-mer covering toxin sequences
Expanding the peptide library with alanine substituted peptides
Light-directed solid phase synthesis of custom peptide microarrays

High number of epitopes recognized by SAIMR antivenom

Figure 1. A-C Venn diagrams of peptides classified to bind antivenom antibodies for each pair of experiments conducted with the same antivenin in two different dilutions. (A) SAIMR Polyvalent Snake Antivenom, (B) VNDE Africa, and (C) Osnabruc Venome Central Africa. (D) Venn diagram of peptides classified as binders for each antivenom. Only peptides identified in both experiments with each antivenom, corresponding to the overlap in Venn diagram in part A-C, are included.

Key residues for antivenom toxin recognition

Figure 2. Examples of B-cell epitope analysis: Type 1 and 2 neurotoxins and dendrotoxins recognized by the SAIMR polyvalent antivenom. The bold profiles above each sequence represent the average score of peptides containing a given peptide. The background represents the average amino substitution effect. When no 12-mer peptide covering a given residue passed the epitope threshold, the residue is colored grey. Dark purple indicates that a residue is of particular importance for antibody recognition.

Antivenoms antibodies bind to functional sites of toxins

Figure 3. Structural presentation of B-cell epitope analysis: (A-B) Short neurotoxin 1 (P01416) from D. polyplexa as an example of a Type 1 neurotoxin. Structure built upon (C) D. fasciculatus 2 (P01218) from D. angusticauda as an example of a fasciculin. The D. fasciculatus 2 is co-crystallized with the human acetylcholinesterase enzyme. Structure built upon (D-F) toxin FS 2 (P01416) from D. polyplexa as an example of an L-iso-calcium channel blocker. Structure built upon (G-H) D. polyplexa (P01397) from D. polyplexa as an example of a calcium channel blocker. Fibrinogen colored according to residue score, where dark red refers to residues with high residue score and blue refers to residues with low residue scores.

Conclusions
Custom-designed high density peptide microarray technology enables parallel automated identification of linear epitopes of snake neurotoxins.

Trend: antivenom antibodies recognize and bind to epitopes at the functional sites of toxins.

Perspectives
Determination of linear elements in snake venom toxin epitopes may provide the basis for:
- Explaining the molecular basis of antivenoms para-specificity
- Guiding next-generation antivenoms based on DNA immunization and immunization with synthetic epitope strings

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