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 elements in epitopes in 82 toxins from four African mamba and three neurotoxic cobra snakes obtained from public databases.

Studying linear epitopes using peptide microarrays

Two approaches were used:
1. Generating an in silico library of 12-residue covering both sequences. Expanding the peptide library with alanine-substituted and other non-native residues.
2. Light-directed solid-phase synthesis of custom peptide microarray.

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 antivenom in two different dilutions. D = SAIMR Polyvalent Snake Antivenom, DE VINA Africa, and E = UNAC 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 best profiles above each sequence represent the average score of peptides containing a given peptide. The blue background represents the average alanine substitution effect. When no 12-mer peptide covering a given residue passed the epitope threshold, the residue is colored gray. 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. polylepis as an example of a type 1 neurotoxin. Structure built upon: (F) Fasciculin-2 (P00128) from D. angusticeps as an example of a dendrotoxin. The Fasciculin-2 is co-crystallized with the human acetylcholinesterase enzyme. Structure built upon: (F) Tom P5-2 (P01416) from D. polylepis as an example of an α-lipopeptide calcium channel blocker. Structure built upon: (E) E-vitamin D3 (P01397) from D. polylepis.

Conclusions

Custom-designed high density peptide microarray technology enables parallel automated identification of linear elements of epitopes in 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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