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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

Type 1 α-neurotoxins

Type 2 α-neurotoxins

Key residues for antivenom toxin recognition

Antivenoms antibodies bind to functional sites of toxins

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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References


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Figure 1. A-C Venn diagram of peptides classified to bind antivenom antibodies for each pair of experiments conducted with the same antivenom in two different dilutions; (A) SAIMR Polyvalent Snake Antivenom, (B) VNC Anti-A (DE), and (C) VNC 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.

Figure 2. Examples of B-cell epitope analysis: Type 1 and 2 α-neurotoxin and dendrotoxin recognized by the SAIMR polyvalent antivenom. The best profiles above each sequence represent the average score of peptides containing a given peptide. The dark background represents the average amino acid 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.

Figure 3. Structural presentation of B-cell epitope analysis: (A-B) Short neurotoxin-1 (P01415) from D. polyxena as an example of a type 1 α-neurotoxin. Structure built upon: (C-D) Fasciculin-2 (P01218) from D. angulatus as an example of a fasciculin. The Fasciculin-2 is co-crystallized with the human acetylcholinesterase enzyme. Structure built upon: (E-F) Tox BS-2 (P01514) from D. polylepis as an example of an α-neurotoxin. Structure built upon: (G-H) Dp-α-latrotoxin Dpp2 (P03529) from D. polylepis as an example of a dendrotoxin. The coloration indicates that a residue is of particular importance for antibody recognition. When antibody recognition score, when dark-red refers to residues with high residue score, and blue refers to residues with low residue score.

Figure 4. Study design for epitope analysis: Treatment groups: (A) Group 1 (D) Group 3, with a dummy control group (C). Immunization of mice with synthetic peptides contains a given peptide or a synthetic control peptide. Immunization the mice with synthetic peptides and polyvalent antivenom. Serum from mice was applied in ELISA experiments with the synthetic peptides and the C3 fraction of SAIMR polyvalent antivenom. The anti-sera is captured by immobilized synthetic peptides. The captured anti-sera is detected with a peroxidase-conjugated secondary antibody. The peroxidase reaction is monitored through the formation of tetramethylbenzidine (TMB) which is measured spectrophotometrically.