BIO_10 - Consensus serineprotease toxin design as antigen and cross-immunization combined strategy for generation of broadly binding antibodies

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Introduction: Next generation antivenoms are a strategy based on monoclonal antibodies and/or small molecules to treat snakebite envenoming, yet broadly binding antibodies are needed for it to become economically competitive. Consensus antigen and cross-immunization techniques have already been shown to promote a broader immune response, and this is particularly interesting for multi-isoforms snake toxins, such as serineproteases (SVSP).

Objectives: In this work we evaluated whether a chimeric serineprotease based on the consensus sequence of serineproteases from *B. jararaca*, and in combination with cross-immunization using native SVSPs could be used as a strategy for development of broadly binding antibodies.

Methodology: Using bioinformatics, we calculated a consensus sequence of seven isoforms of serinoproteases, identified conserved regions and epitopes, and transiently expressed the chimeric consensus serineprotease (SVSPq) on Expi293F cells. Later, we immunized Balb/c mice with SVSPq, native serineproteases (SVSPn) purified from *B. jararaca*, or a combination of both, and evaluated the immune response using ELISA and Western Blotting.

Results: Through immunoinformatics analysis, we identified the conserved regions in *B. jararaca* SVSPs using the AL2CO entropy measure, we calculated a consensus sequence and created a chimeric SVSP, which we included in our analysis. We identified several conserved epitopes, ranging from 70%-100% similarity between isoforms, those epitopes are present in our construct. After our immunization protocols, our results shows that the sera from mice immunized with SVSPq bind to native serineproteases, and mice immunized only with SVSPn bind to SVSPq, demonstrating the existence of conserved epitopes in our construct. The cross-immunized groups developed a stronger immune response against both. Next we aim to isolate the coding sequence of those antibodies, to develop a recombinant antibothropic venom.

Conclusion: Together, these results show that the consensus antigen strategy could be used for next-generation antivenom development, and the combination with cross-immunization could further improve the discovery of broadly binding/neutralizing antibodies.

Keywords: Ophidism; Antivenom; Monoclonal Antibody; Antigen Design; Serineprotease