

## **Immune response against *Bothrops diporus* venom pre-treated with Na<sub>2</sub>EDTA formulated with a nanostructured (Coa-ASC16) and CpG-ODN sequences**

In South America, most snakebites are caused by genus *Bothrops*. The *B. diporus* venom is composed mainly of metalloproteinases (SVMPs) responsible for local effects such as hemorrhage, edema, myotoxicity and systemic bleeding. The only treatment for snakebite is antivenom, produced by immunizing animals with snake venom using Freund's adjuvant, which causes local damage at the injection site and affects the welfare of producer animals. To address this issue, *B. diporus* venom was treated with Na<sub>2</sub>EDTA and used as immunogen in combination with CpG-ODN/Coa-ASC16 adjuvant. Previous works demonstrated that CpG-ODN/Coa-ASC16 adjuvant causes very few local reactions when used with other antigens. For this proposal, *B. diporus* venom (1.8 mg) was incubated with Na<sub>2</sub>EDTA (0.2 M, BdV/Na<sub>2</sub>EDTA) for 1 h at 37°C and then it was subjected to Sephadex G-25 chromatography to remove the excess of chelator. Likewise, *B. diporus* venom without chelating agent (BdV) underwent the same process. In both samples, the effective inhibition of SVMPs using azocasein as substrate was determined. CF-1 mice (5/group) were immunized subcutaneously on days 0, 15 and 30 with BdV or BdV/Na<sub>2</sub>EDTA (7-30 µg) formulated with CpG-ODN/Coa-ASC16. On day 45, the final bleeding was performed and sera were obtained for antibody detection. The enzyme-linked immunosorbent assay results indicated that anti-BdV and anti-BdV/EDTA exhibited similar antibody titers ( $\sim 2.56 \times 10^4$ ) against Bothropic venom. Western Blot analysis revealed that the anti-BdV/EDTA serum recognized the main venom proteins (15-150 kDa) similarly to the anti-BdV serum. Finally, both experimental sera displayed neutralizing abilities against the proteolytic, indirect hemolytic, and coagulant activity tested in vitro. These findings suggest that Na<sub>2</sub>EDTA does not impair protein immunogenicity, and BdV/Na<sub>2</sub>EDTA together with CpG-ODN/Coa-ASC16 adjuvant was an appropriate immunogen since the animals immunized with it showed an adequate immune response to *B. diporus* venom similar to that of animals immunized with venom without an inhibitor.