CLONING, EXPRESSION AND SCREENING OF TOXINS

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In the current year, we focused our studies on the screening of many venoms and toxins, aiming to identify molecules with potential pharmacological use. Amongst the assayed molecules was bothropstoxin-1 (FIG.1), a potent myonecrotic snake toxin. In tests with several monoclonal antibodies, raised in our laboratories, we identified four putative neutralizing antibodies. Considering that the mechanism of action of the toxin has not yet been totally explained, these antibodies may represent a powerful tool to further investigate the structural motifs involved in target recognition and onset of cell-specific necrosis. Also, we cloned and expressed a fully active recombinant bothropstoxin-1 in *Escherichia coli*. We believe that using both the antibody and a site directed mutagenesis approach, we should be able to get some insights on the mechanisms involved in the myotoxic activity. Also, in collaboration with Dr. Moura da Silva, we performed a screening on a wide array of venoms with antibodies against jararhagin, an extremely potent hemorrhagic toxin. We identified several not yet described putative hemorrhagic toxins in many elapid snake venoms, a fact that may be relevant from the clinical point of view. Indeed, these proteins are known to be involved in the severe coagulopathy observed in snake bitten patients.
In collaboration with Dr. José Carlos de Freitas, an activity screening was performed with marine animal toxins, resulting in the isolation of substances with potent bacteriostatic activity against both gram-negative and positive organisms. These substances are now been purified for further characterization and in order to investigate their potential as new generation antibiotics.

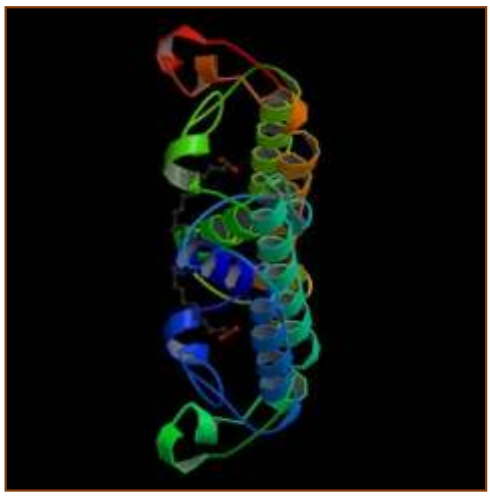

FIGURE 1 - Tri-dimensional structure of bothropstoxin-1

GAMMA RADIATION STERILIZED PARASITES FOR VACCINE DEVELOPMENT

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The irradiation of proteins and other biomolecules leads to modifications of these substances, and this feature has been used to inactivate and attenuate molecules as well as to sterilize many kinds of products. Recently, we found that the irradiated protein could be selectively incorporated to the cells, due to specific receptor for oxidized protein, the scavenger receptors. This increased uptake could also result in better antigen presentation and high immune response, either humoral, as demonstrated with purified crotoxin or cellular, as recombinant M.leprae rp18 heat shock protein. Toxoplasmosis, caused by Toxoplasma gondii, is a disease that affects half a billion of people around the world, due the high level of environmental resistance of the oocyst of the agent, produced in infected kitten stools, and the presence of cysts in meat from livestock, usually lambs and pork. Despite the fact that the infection in humans is generally benign, due to large numbers of infected people, there is an enormous social burden related to eye disease. Our scope was to test the use of irradiated Toxoplasma gondii as a vaccine candidate. The results indicate that under controlled conditions we were able to obtain viable parasites that were unable to reproduce both in vivo and in vitro. When the immune response of animals immunized with this preparation was assayed, the results were similar to the one observed with animals infected with native parasites, both antibody production, cellular proliferation induced be external antigen, and also cytokine production similar as chronic disease. These results may provide the basis for vaccine development, as the immunized animal presented very low disease when challenged by mouth, as usually the infection begins in man. Now we are testing those vaccines by oral route, which induced significant intestinal immunity, with protection similar to parenteral vaccine, a very promising finding. Using the same approach, we are now testing radiation as an attenuating agent for Leishmania, the causative agent of leishmaniasis, and another epidemiologically relevant parasitic disease affecting millions of people.