

A proposed pilot study of ophidian DNA vaccinology

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Surviving snakebite: Active Immunization of Tribes and Agricultural People Throughout the World with an Ophidian DNA- Based Vaccine.

By Tim Friede

Every year over 5 million people are affected from snakebites worldwide : of them, 125,000 die - 100,000 in Asia, 20,000 in Africa, and 5,000 in the Americas (4, 5). And among survivors, many are disabled because of venoms that damage fingers, hands, arms, and legs. Because of location, lack of medical support, or no refrigeration, it's very difficult to develop a vaccine that suits the needs of these situations. Currently the only form of a cure is antivenom. The focus of my project is to cut antivenom out of the picture by actively immunizing tribal and agricultural people around the world with an ophidian DNA-based vaccine. This approach will save many lives; many fingers, arms, and legs; and time to get to antivenom if needed.

In the past, solutions included two forms of active immunization, one in a lab setting and one in a field setting. Lab setting experiments were done by Bill Haast, Charles Tanner, Harold Mierkey, Ray Hunter, Herschel Flowers, and myself. The methods included pure venom injections by Haast, Mierkey, and myself and dried venom by Hunter and Flowers. Tanner's immunization used purified dried venom along with a toxoid (3).

The pure venom injection protocol that Mierkey and I use is based on a 6-mg maximum injection that yields very high IgG antibody titers (mambas, higher than 5 mg/ml) (3); however, the body can't handle 6 mg from any genus (the cobra complex [*Naja* spp.] is a prime example because of the strong cardiotoxic activity. Only partial protection would fall into this category (2.5 mg/ml); and antivenom might be required. This form of active immunization works very well but is not suited for field trials due to the danger of IgE anaphylactic shock exposure and the requirement for refrigeration.

Toxoids and dried venoms would also fall into some of these categories. 2 field trials using a toxoid solution - one in 1986 in Burma and a larger one from 1965 to 1967 in the Amami/Ryukyu Islands - had fair results. Here again, the lack of refrigeration would pose a major problem.

At the end of 2005, I took a small break to gather my thoughts, to look at what I've done, to study what others had done, and to decide if moving forward with this project was worthwhile. At this point I was studied twice, once by Dr. Morinaga in Japan and then at Kagen Allergy Clinic in Wisconsin (3). My research was presented on by Dr. Steve Kagen at the American College of Allergy, Asthma & Immunology (ACAAI) in New Orleans and by Dr. Toriba in Africa at the 5th World Congress in Herpetology (WCH) in 2005 (3). I also collected much in the way of peer review from many immunologists,

scientists, and doctors who were kind enough to help with their thoughts on this matter (Dr. Anthony Tu, Dr. Steve Kagen, Dr. Ramasamy Muthiah, Dr. Gauthier, Dr. Wasil Kahn, Dr. Stanley A. Plotkin at Aventis, Prof. Franc Gubensek, Prof. Findlay Russell, Dr. John Harris, Dr. Pele Chong, Dr. Shivaji P. Gawade, Prof. Daniel Dietrich, Prof. Paul Potter at University of Cape Town, Dr. Jim Wallis, Dr. Michihisa Toriba at the Japan Snake Institute, and Dr. Ming Tu). Their aid and advice were extremely helpful in my research.

When I wrote my book on the vaccine for Dr. Toriba, I added a small paragraph by Dr. Robert Harrison at the School of Tropical Medicine in Liverpool about DNA vaccines with snake venom using mice (3). Many ideas jumped out at me : the strong immune responses that could be generated; bypassing the requirement for refrigeration; easier to reproduce than other vaccines; safer than most vaccines. So, the advantages are obvious for the primary and secondary cure. I also ran across another positive feature by Powdermed, who developed a gene gun that's being used for influenza and hepatitis that requires less dosage, no refrigeration, easier transportation, easier storage, and minimum pain. This approach, I felt, was the perfect fit for what was needed for this project. The above features are essential for the effectiveness of the ophidian DNA-based vaccine; but two major questions remain: can it produce high titers and how many shots are needed in the course of a year to maintain acceptable immunity?

I ran across an amazing website by Dr. Daniele Focosi (<http://www.mi.interhealth.info>), in which I found much of the information I couldn't find anywhere else. Upon contacting him regarding my plan, he was very supportive and offered a perfect plan of attack. He felt the only solution was the DNA vaccination route, because of the many positive features. DNA vaccinology is a safer route to take; creating snake-specific DNA vaccines wouldn't be a major challenge since most toxins have been characterized and sequenced so that the respective encoding DNA fragment can easily be engineered in vitro (2, 5). Dr. Focosi recommended I focus on contacting DNA companies that specialize in this area : they could eventually use me in pilot studies with snakes I don't use for immunization. Then, if that works out, the vaccine would be taken to the field for human trials (2).

SIMPLIFIED OPHIDIAN DNA VACCINE PROTOCOL (Proposed)

Take a blood sample, separate out the sera and freeze.

Day 0 : 2 x 1 mg total pDNA delivered I.M. (deltoid)

pDNA via tattoo (each thigh).

Day 14> repeat both.

Day 28 > same

Day 42> same

Day 56> repeat both and draw a blood sample, separate out the sera and freeze

Day 70> same.

Establishing a titer by different methods and using further boosters might be in order, along with a challenge test (1).

We have made much progress up to this point, and things look very positive. Dr Focosi's expertise with DNA vaccines will be a valuable part of this trial, and he agreed to help with medical parameters during the trial. A private company has offered to help with DNA formulation.

The resultant tragedies of death and disability can be greatly reduced by the application of a DNA-based vaccine; or even a partial vaccine. Disseminating this information on the DNAvaccine.com website (<http://dnavaccine.com/>) will hopefully raise awareness of this serious medical problem in Third World countries, gain supportive DNA protocol knowledge with human field trials, and raise funds for human field trials.

If you're interested in supporting our project with ideas or funds, please feel free to contact me directly.

Sincerely,

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References

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