HUMAN IMMUNIZATION, TO ACQUIRE ACTIVE IMMUNITY AGAINST SNAKE VENOM

History

It has long been established that animals can be actively immunized against snake venom by repeated parental injections, with increasing doses of venom (Sewall, H., 1887.) Active immunity against snake venom has also been attempted and demonstrated in humans using toxoid derived using the venom of the Australian Tiger snake (*Notechis scutatus*) (Wiener, S., 1960.) A large-scale immunization trial was also implemented using a toxoid of the venom from the Habu (*Trimeresurus flavoviridis*) (SAWAI et al. 1969). The most remarkable case of active immunity in humans is of one William E. Haast of the Miami Serpentarium. He originally obtained immunity using venom from the Cape Cobra, (*Naja nivea*) then later included venom obtained from the Indian Cobra, (*Naja naja*) and King cobra (*Ophiophagus hannah*). His case demonstrated cross-resistance between venoms of different species. He subsequently was bitten by species other than was used in the inoculations and suffered little or no systemic reactions. The most remarkable followed after being immunized with the Cape cobra (*Naja nivea*). He was bitten by the Blue krait (*Bungarus caeruleus*) and survived without any antiserum. This case unlike most other cases of human immunization against snake venom involved using venom that was not detoxified (Haast, W.E, Winer, M.L. 1955). Later other venoms were included into his inoculations. Presently more than thirty venoms are used in his booster shots. He has subsequently survived more potentially lethal bites than any other human. These bites included some of
the most lethal species. The Tiger snake (*Notechis scutatus*) Russell's viper (*Daboia russelii*) and Saw scale viper (*Echis carinatus*) are a few of the many notable species he has survived bites from. He is presently ninety four years old and in good health.

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(Haast, W.E., Nancy Miami Serpentarium 2005 personal communications)

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Case

This case, before us now, is another using venom that is not detoxified. This case involved
a male subject, the author, who is of good health and condition. The immunization was
carried out as a prophylaxis in case of envenomation, while engaging in venom extraction. Liquid,
non-lyophilized venom was used for convenience. All doses are in the liquid natural form.

Detoxification was not attempted in order to eliminate any epitope or antigenic change.
No adjuvant was added. Prior to doses of .01 ml of pure undiluted venom, dilutions were
made using bacteriostatic water with 0.9% benzyl. Inoculations were administered
by intradermal injections. The course of immunization was carried out from September 2001 to March 2005. Inoculations were given one week to one month apart. On two accounts there where delays between inoculations of a period of two months. Immunization commenced using venom from Egyptian cobra (N. haja) and Forest cobra (N. melanoleuca) at a dosage of .001 ml. These venoms were rotated between inoculations. Later Cape cobra (N. nivea) venom was used as a substitute to replace other naja venoms. This variation from the original immunization schedule was done because of the known toxicity of N. nivea venom.

It being the most toxic of all cobra venoms (U.S. Department of the Navy, Bureau of Medicine and Surgery, POISONOUS SNAKES OF THE WORLD, 1991). It is the hypothesis of the author, that because of its toxicity, N. nivea would provide the best immune protection against most other similar naja venoms. The use of N. nivea venom commenced after the inoculation doses of the other naja venoms were .01 ml. At this stage of the immunization when the use of different, but similar venom was implemented, a lower dose of .005 was used initially. In this case, immunity obtained using the other naja venoms proved affective in that no
substantial reactions occurred following initial and subsequent injections with *N. nivea* venom.

Due to the absence of systemic reactions and only minor local reactions, these doses were doubled with each inoculation until the previously used dose as with the other venom was obtained. This stage of immunizations continued with these venoms until a dose of .02 ml of undiluted liquid venom was used. During this course no serious systemic effects were experienced. General malaise was experienced during earlier inoculations. On a few occasions pyrexia was also experienced.

Systemic effects decreased with repeated injections of the same amount. Local reactions always consisted of an immediate wheal, erythema, ecchymosis, and subsequent swelling distal to injection site. Edema sometimes encompassing the entire limb and usually subsided within 2 days. Initial and higher doses always resulted with local necrotic ulcerations, acute cellulitis and subsequent holes when the necrotic tissue was debrided and/or sloughed from injection site. The necrotic ulcerations and cellulitis would also progressively show signs of healing within 2 days. During the course of these inoculations, it was also noted that after lapses of two months, the only noticed reduction in immunity or immune responses were noticed in these local effects. Shorter intervals between inoculations produced less of a
local reaction. Systemic involvement remained more consistent, with no changes in reactions after these 2-month lapses. The course of immunization using venom of the Eastern Green Mamba (*Dendroaspis angusticeps*) was started when the dose of naja venoms were .02 ml. The same initial dose, as used with the others, of .001 ml was used. The effects of this of *D. angusticeps* venom proved substantially different, than the naja sp, used with the previous inoculations. It proved different with its extremely rapid onset of symptoms. This was possibly due to the facilitating ability of the dendrotoxins and / or to its extremely light viscosity. Snakes belonging to the *Dendroaspis* genus have a particular dreaded reputation. This is partly due to this rapid onset of systemic symptoms. Prior to the development of mamba antivenoms, there where difficulties experienced in their development. The animals often died, as a result of the venom acting so rapidly (*Krush, Haast Cobras in His Gardens 1963*). Initially, in each case of these earlier inoculations, using *D.A.* venom, numbness and tingling sensations of the lips, mouth and brow was experienced immediately upon injection. Sore throat and muscular aches later followed these symptoms of the
earlier inoculations. Some of these earlier doses which resulted with notable reactions, would persist into the next day with symptoms similar to those following other intoxications or flu-like symptoms, i.e. headaches, sore muscles and joints.

The symptoms lessoned with subsequent inoculations of the same amount. However symptoms would return with increased doses. Inoculation doses, up to this point, where; .001, .002, .003, .004, .005, .006, .007ml. When inoculation doses of this venom were .008 ml, symptoms came on quickly, like with a “rush”, then would begin to subside within 2 hours. During these occasions it was required to lie down in bed. In two accounts, recorded blood pressures were noted with a decrease in systolic and diastolic pressure. On one of the inoculation using the dose of .008, the BP of 78/40 was recorded initially. BP then fluctuated, but gradually increasing and returned to within normal parameters within one hour. Pulse rates increased considerable after injections initially along with the feeling of a “flush” hot sensation. Pulse rates as high as 105 per minute were recorded. The pulse rate would then drop to as low as 40 per minute. The advent of these symptoms were within two (2) minutes following injections. Vital signs would return to normal parameters within 2 hours. The reversal of these rapidly developed symptoms were as notably felt as
with the onset. This is interpreted as a positive immune response. The subsequent dosage following the above inoculation and adverse symptoms was reduced to the last previous dosage without these symptoms, so that the previous dose, in this case .007 ml. was repeated. Tonic muscular spasms associated with flexion and extensions while walking, resulting with extra movements while taking steps, were also experienced. These spasms persisted after all vital signs returned to normal limits, then would gradually return to normal. However sensations and reflex actions attributed to this persisted into the next morning. These spasms were possibly indications of the fasciculins or dendrotoxins, that facilitate the release of acetylcholine, without endogenous regulation and control. Initially this venom did prove difficult to use as an antigen for inoculations because of this rapid onset of symptoms and effects. However, once the dose of .01 ml of undiluted was reached, all subsequent increases of dosage were uneventful. No remarkable deviations of vital signs were experienced following subsequent injections of higher doses. It was after a dose of .04 ml. undiluted D. angusticeps venom that dose increases of venoms; N. nivea and D. angusticeps in increments of .01 until a dosage of .15 ml undiluted venom with no systemic effects. At this dose of .15 ml, D.A. venom caused only erythemas and edema of
site, with no significant signs of systemic changes. No cellulitis or necrotic effects were noted following any inoculations using venom of D. angusticeps, only an immediate wheal followed by erythema and subsequent swelling was observed in the immediate injection site. A challenge dose of .15ml N. nivea venom caused no systemic effects, only the previously described local necrotic effects. The effects on the tissues in the immediate areas did appear dose dependant, but only to a point. A dose of .15 ml caused only slight increase in the local reaction to that of .05 ml. This is consistent with the observations of Wiener (Wiener, S. 1960). The venom of the Western Green Mamba (D. viridis) is 3 to 4 times more lethal than D.A venom. (Harvey et al, 1984 J. Toxicol) However, inoculations of D.V with venom doses of .01, .02, .04, .08, 10, .15ml., given one week a part, subsequent to D.A. immunization, were uneventful. It remains unknown what effects an initial higher dose of D.V. venom and what degree of immune protection would have been provided following D.A. immunization. However, it is evident with the accelerated immunization using D.V. venom without ill effects are an indication of substantial protection. This is possibly because the toxic constituents of D.V. venom are similar to the
combination of *D.A.* and *N. nivea* venom. *D. A.* venom does not contain potent post synaptic neurotoxins as found in both *D.V.* and *N. N.* venom. (Harvey et. al, 1984 J. Toxicol) The absence of notable systemic symptoms following a dose of .15ml undiluted venom from these species, as well as the rapid rate at which symptoms subsided, following earlier inoculations is evident of some substantial immunity. The unsuspected adverse reactions, as noted following *D.A.* dose .008 is an example of difficulties that can be experienced during immunizations of snake venoms that have not been attenuated.

On the 21st of April 2005, a bite was sustained from a four foot Eastern diamondback rattlesnake, *Crotalus adamanteus*. The bite was to the right middle finger with both fangs and a scratch to the index.
hand and wrist, which was less than double the normal size. Only the wound area was
discolored, and with small blebs. No antivenin was administered. Laboratory test; CBC,
Fibrinogen level and prothrombin time revealed no gross abnormalities. Discharge from
the
hospital was the same night. It appears evident that despite the known marked differences
between crotalid and elapid venoms, there was substantial immunity demonstrated in this
case.

*This bite sustained from the *Crotalus adamanteus* is inconclusive as an indication
of an immune response. Even so it should not be over looked entirely. It should stimulate
curiosity. Booster inoculations are continued on a monthly basis. The author is confident
and
predicts that the unique properties of *Dendroapsis* venom will hold some significance
regarding
the further understanding of the generation, transmission and reception of acetylcholine.
There
also appears to be evidence, here, which suggest an immunopotentiation between different
snake venoms as antigens. Further investigations could reveal more information regarding
the immune responses between antigenic toxins and antibodies produced heterologously by
inoculation of different venoms. This phenomenon may be due to the affinity at which
different
antibodies bind to antigens exogenous to the production of that specific antibody or further
research may reveal new information regarding antibody production and differentiation of
undifferentiated B cells. This needs further investigation. It should be made known that the
The foregoing paper is not intended as an account of any study, but rather of the author's own immunization schedule used and of the observations of the effects experienced during its course.

The author wishes that this information be made available to any and all researchers that may find interest.

The author must emphasize that the risks involved in such a procedure as the one described above would normally be unwarranted. The reactions as indicated are unpredictable and vary.

No standardized method has been determined and the probability of extreme and serious consequences great.

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