New techniques in antivenom production and active immunization against snake venoms

R. D. G. Theakston

Alistair Reid Venom Research Unit, Department of Tropical Medicine, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, UK

Standard methods of commercial antivenom production are costly both in terms of animals, their maintenance, the use of large amounts of expensive venoms and man-hours. The basic technology, which involves the hyperimmunization of horses, bleeding and subsequent purification of the plasma immunoglobulin G (IgG) using salt precipitation and pepsin digestion, has not advanced significantly for many years. In most cases monospecific (animal immunized against a single venom) or polyspecific (animal immunized against a mixture of different venoms) antivenoms are used in developing countries where snake bite is a serious problem. Funds for purchase of such drugs are of necessity limited; this can therefore result in relatively low profit margins with associated frequent inadequate quality control and substandard products.

Liposomal immunization

Currently new methods of animal immunization are being developed, one involving the use of specially treated membrane-stabilized liposomes incorporating venom which stimulates a rapid and sustained high-titre protective antibody response in experimental animals (mice, rabbits and sheep) following a single subcutaneous or intravenous immunizing injection (New et al., 1984, 1985; Theakston et al., 1985). The immune response using this procedure can be intensified by incorporating immunostimulants such as Escherichia coli endotoxin or lipid A into the venom/liposome system (Laing et al., 1986, 1988). Antivenoms developed using this or related methods should result in the development of cheaper and more avid antivenoms for use in developing countries.

The liposome technique also has potential application as a screening test for horses suitable for immunization. For example, antivenom producers in Brazil have problems over selection of suitable horses for immunization against the venoms of Crotalus durissus subspecies; many animals either do not respond at all or are poor responders to standard immunization procedures, possibly due to some form of immunosuppression (Bolanos & Cerdas, 1980). Horses could be screened by inoculating them with a test dose of this venom incorporated into liposomes. Animals which become positive by enzyme-linked immunosorbent assay 2 weeks after the injection should be suitable for the full immunization protocol.

Active immunization of man has been attempted with some success (Sawai et al., 1969; Haast & Wiener, 1955; Wiener, 1960, 1961; Flowers, 1963), but Rosenfeld (1971) reported that it did not provide real protection due mainly to the short-lived antibody titres conferred; immunization would not work unless the challenge bite occurred soon after the end of the immunization procedure. Investigations are in progress to study the possibility of active immunization of man using venom/liposomes administered both parenterally and orally to experimental animals. Laing et al. (1988) demonstrated a raised serum IgG response in experimental mice following oral administration of Echis carinatus venom incorporated into specially stabilized liposomes following a post-immunization sub-lethal subcutaneous boost of whole venom. This indicates a priming of the immune system following oral immunization. New et al. (1985) also reported a direct serum IgG response in mice following oral administration of venom liposomes. These workers concluded that although the serum antibody titre was not high enough to confer direct protection, the observation of such a response suggests the generation of a considerable degree of intestinal-mediated immunity.

Enrichment of commercial antisera

Russell et al. (1985) considered that, because of the difficulty in determining the amount of commercial antivenom required to mitigate the clinical effects of a venom, often large amounts of horse serum protein need to be given to a patient, with an associated increase in the incidence of serum reactions. Specific antibodies contained in commercial (Wyeth) antivenom were purified using polyacrylamide gel affinity columns to which were adsorbed a range of rattlesnake venoms. The purified IgG antibodies were more effective in neutralizing lethal, haemorrhagic, coagulant, platelet-aggregating, necrotizing and neuromuscular blocking effects of the venoms than the commercially available Wyeth antivenom. It was suggested that the superior properties of the purified IgG may be related to the enrichment of specific antibodies by elimination of other (non-useful) proteins. The incidence of serum reactions was also reduced. The elimination of the expensive ammonium sulphate precipitation stages in preparation also could decrease the loss of neutralizing IgG. According to Russell et al. (1985), antivenom prepared using these techniques is currently being produced for clinical trial, and further studies are in progress on the F(ab) fragments prepared from the cleavage of the whole IgG molecule. Likewise the supplementation (four-fold increase in anti-procoagulant titre) of a monospecific antivenom with anti-Arvin led to a significant increase in the anti-coagulant
activity of a monospecific Galloselasma rhodostoma antivenom in Thailand (WARRELL et al., 1986).

Monoclonal antibody technology

The development of avid monoclonal antibodies active against pathological venom fractions may be a further possibility. The mode of action of antivenom (i.e., how antibodies neutralize venom toxins) is not known, and probably differs from one toxin to another. To improve antivenom serotherapy, it is critically important to determine whether antibodies interact at the toxic or enzymatic site of the molecule or at an unrelated antigenic site, and which isotype of immunoglobulin is most effective in its neutralizing activity. MENEZ (1985) considered that, in the case of elapid venoms, one monoclonal antibody could neutralize the main neurotoxin. Recent work by BOULAIN et al. (1982) and BOULAIN & MENEZ (1982) suggests that in the case of the α-neurotoxin of Naja nigricollis venom, a monoclonal antibody with an epitope localized at a distance from the acetylcholine binding site can effectively interact with venom-acetyl choline binding, either by inhibition of binding or by dislocation of the association when binding has already occurred (MENEZ et al., 1986). The latter mode of action is consistent with the kinetics of antivenom therapy after neurotoxic poisoning in humans, when the serum remains beneficial for several days after snake bite. The major implication of the results obtained in this model system is that a well-chosen polypeptide fraction of a toxin could, when linked to a suitable carrier/adjuvant, be used for immunization to prepare antivenom without the usual toxic side effects. Such a molecule could possibly be used for preventive immunization of individuals at high risk. Work in this laboratory is currently concentrating on the development of 'useful' monoclonal antibodies against the α-neurotoxins of N. kaouthia venom (KARLSSON et al., 1971) and an in vitro assay system is also being developed for the study of elapid venoms, and isolated neurotoxins, which will be useful for studying the neutralization by both monoclonal antibodies and antivenoms in general. The system involves the use of non-solubilized human acetylcholine receptors instead of the more conventional technique which uses Torpedo acetylcholine receptors (WEBER & CHANGEUX, 1974).

The use of such avid monoclonal antibodies, either as supplements to currently available antivenom or as separate therapeutic entities, could therefore have real application in envenoming by elapids, such as N. kaouthia, where up to 450 ml of antivenom is often necessary to reverse or eliminate the symptoms of systemic envenoming (VIRIVAN et al., 1986). An avid anti-neurotoxin monoclonal antibody supplement may also result in much smaller volumes of antivenom being required for treatment of neurotoxic envenoming.

The application of monoclonal antibodies for treatment of vipersine envenoming is less hopeful, as a multiplicity of toxins are involved in the production of systemic signs. A 'cocktail' of monoclonal antibodies essentially containing anti-haemorrhagic and anti-coagulant components would therefore be necessary. It is also possible that these high molecular weight components may have several antigenic determinants and it may be unlikely that a single antibody could neutralize their activity (PEREZ et al., 1984), although conversely IDDON et al. (1985) and PUKRIT-TAYAKAMEE et al. (1983) have reported monoclonal antibodies which are capable of significant neutralization of these components. Additionally, because of the derivation of monoclonal antibodies from mouse myeloma cells, there may well be ethical objections to their use in therapeutic treatment. Currently it is thought that the main potential of monoclonal antibodies is in the isolation of pathological venom fractions from whole venom by affinity chromatography or by recovery of recognized protein bands from electrophoresed gels (IDDON et al., 1988). These fractions could then be used for the subsequent production of highly specific antitoxins, probably by incorporating them into the liposomal immunization system. According to MENEZ (1985), another approach may be the chemical analysis of the toxin fragments that correspond to the antigenic areas which bind specifically to efficiently neutralizing antibodies.

Recombinant deoxyribonucleic acid (DNA) technology

TAYLOR et al. (1986) used monoclonal antibodies (IDDON et al., 1988) to determine that the optimum time to remove gland tissue for extraction of ribonucleic acid (RNA) for use in the production of a cDNA library was on the third day following 'milking' of E. carinatus; at this time maximum synthesis of venom proteins was occurring. Currently work is in progress on the production and characterization of cDNA libraries to determine whether useful antigens (e.g. haemorrhagins, procoagulants, etc.) have been produced. Recently TAMIYA et al. (1985) cloned the cDNA encoding a curaremotic toxin precursor with the aim of producing mutant proteins with single mutations by means of site-directed mutagenesis (PIELAK et al., 1985).

Conclusions

There is an urgent need for improving the neutralizing potential, and decreasing the cost, of antivenoms for alleviation of the major snake bite problem which occurs in many parts of the rural tropics. In many isolated areas where there is a high incidence of, and mortality due to, snake bite, there exists a good case for active immunization of the communities at risk. One of the main aims of this article is to draw attention to these serious but frequently underestimated problems and to suggest how they may be overcome.

References


