

Production of Human Antibody Fragments Binding to Melittin and Phospholipase A2 in Africanised Bee Venom: Minimising Venom Toxicity

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Abstract: The hybrid created from the crossbreeding of European and African bees, known as the Africanised bee, has provided numerous advantages for current beekeeping. However, this new species exhibits undesirable behaviours, such as colony defence instinct and a propensity to attack *en masse*, which can result in serious accidents. To date, there is no effective treatment for cases of Africanised bee envenomation. One promising technique for developing an efficient antivenom is the use of phage display technology, which enables the production of human antibodies, thus avoiding the complications of serum therapy, such as anaphylaxis and serum sickness. The aim of this study was to produce human monoclonal single-chain Fv (scFv) antibody fragments capable of inhibiting the toxic effects of Africanised bee venom. We conducted four rounds of selection of antibodies against the venom and three rounds of selection of antibodies against purified melittin. Three clones were selected and tested by enzyme-linked immunosorbent assay to verify their specificity for melittin and phospholipase A2. Two clones (C5 and C12) were specific for melittin, and one (A7) was specific for phospholipase A2. In a kinetic haemolytic assay, these clones were evaluated individually and in pairs. The A7–C12 combination had the best synergistic effect and was chosen to be used in the assays of myotoxicity inhibition and lethality. The A7–C12 combination inhibited the *in vivo* myotoxic effect of the venom and increased the survival of treated animals.

The crossbreeding of African bees and European bees has resulted in the creation of the hybrid species known as the Africanised honey bee [1]. Despite the advantages that it has brought to apiculture, this new species (*Apis mellifera*) is considered extremely aggressive and is associated with various types of accidents, including mass attack. These bees, popularly known as “killer bees”, already dominate Central and South America, as well as part of North America [1–3].

Martins *et al.* [4], studying children under 15 years of age in southern Brazil, reported that accidents involving bees are the second most common type of envenomation among individuals in that age bracket, accounting for 25.9% of all such accidents, making them even more common than spider bites (22.8%), scorpion stings (1.6%) and snake bites (1.1%). In São Paulo, more than 1000 accidents involving bees are reported every year, with a large increase between 1998 and 2005. In 2010, there were 1030 such accidents, resulting in four deaths [5].

The venom of *A. mellifera* is a complex mixture of toxic chemicals whose concentration and composition can present seasonal variations. It is primarily composed of group III secretory phospholipase A2, hyaluronidase, lipases, phosphatases, apamin, and mast cell–degranulating peptide [6,7].

Melittin, which is derived from promelittin protoxin, is a cationic, amphiphilic polypeptide composed of 26 amino acid residues [8,9]. It accounts for 50% of the total protein content of the venom [10] and is highly water-soluble but also capable of associating with natural and artificial membranes [8,11]. Melittin has a cytolytic effect, being considered a direct haemolytic factor. It can also act synergistically with phospholipase A₂, releasing arachidonic acid in rat PC12 cells [12], luteinising hormones from pituitary cells [13], lysosomal enzymes from rabbit neutrophils [14] and insulin from islet cells [15].

Bee envenomation can produce a syndrome in which there are severe disturbances such as respiratory depression, acute kidney injury, severe anaemia, rhabdomyolysis, tachycardia, headache, vomiting, abdominal cramps and elevated creatinine levels [16,17]. Owing to the high mortality associated with this syndrome, affected patients should be admitted to the intensive care unit [1,3].

The search for an effective treatment for bee envenomation has been the object of many studies. Jones *et al.* [2] produced an antivenom against Africanised bee venom. However, the use of heterologous sera can cause severe complications such as anaphylaxis and serum sickness. One alternative to the development of a new antivenom is the production of human antibody fragments by phage display technology. This technique mimics the strategy used by the humoral immune system to produce antibodies and

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dispenses the immunisation of animals and the construction of a hybridoma [18].

Materials and Methods

Animals and venom. Africanised bee venom was obtained by dissecting the poison sac and compressing it against a capillary coupled to a micrometer. The poison was diluted 1:100 in 0.15 M saline, quantified, aliquoted and stored at -20°C . Africanised bees were provided by the apiary of the Department of Genetics at the *Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (FMRP-USP, University of São Paulo at Ribeirão Preto School of Medicine)* in Ribeirão Preto, Brazil. Swiss female mice (18–22 g), supplied by the FMRP-USP Central Animal Laboratory, were used in determining the median lethal dose (LD_{50}), as well as in tests of myotoxicity and lethality. The study design was approved by the FMRP-USP Ethics Committee on Animal Experimentation.

Library and antibodies. The Griffin.1 library, a phage display library of synthetic single-chain Fv (scFv) phagemid human V-gene segments, was generously provided by Dr G. Winter (Centre for Protein Engineering, Medical Research Council, Cambridge, UK). Horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G was purchased from Sigma (St. Louis, MO, USA). Horseradish peroxidase-conjugated rabbit antibodies against the VCSM13 helper phage and rabbit anti-scFv antibodies were produced as described by Tamarozzi *et al.* [19].

Selection of phage antibodies against Africanised bee venom. For the selection of phage antibodies, a 5-ml vial (Nunc-Kamstrup, Roskilde, Denmark) was sensitised with 50 μg of bee venom, as described by Marks *et al.* [20]. The Griffin.1 phage antibody library (1 ml) was added to the vial, which was slowly rotated for 30 min., after which it was allowed to sit for 90 min. After having been washed with phosphate-buffered saline containing 0.05% Tween-20 (PBS-Tween; Acros Organics, Fair Lawn, NJ, USA), pH 7.2, the remaining phage antibodies were eluted by adding 1 ml of 100 mM triethylamine (Sigma). The vial was slowly rotated on ice for 10 min., during which time 500 μl of 1 M Tris (Sigma), pH 7.4, was added. An aliquot of the phage antibodies (750 μl) was used in the amplification step by infecting *Escherichia coli* TG1 in the exponential growth phase [optical density (OD) of 0.4 at 600 nm]. Phage antibodies to be used in the next round of selection were recovered by precipitation with polyethylene glycol/sodium chloride. At this step, the VCSM13 helper phage was used. There were four rounds of selection.

We used enzyme-linked immunosorbent assay (ELISA) to determine the specificity of selected phages against crude Africanised bee venom, purified melittin and purified phospholipase A2 (Sigma). A 96-well microplate (Corning, New York, USA) was pre-sensitised with 2% milk powder in 50 mM carbonate-bicarbonate buffer, pH 9.6, and allowed to sit for 12 hr at 4°C . The plate was then washed three times with PBS, pH 8.0, treated with 30 μl of 25% glutaraldehyde (Vetec, Rio de Janeiro, Brazil) and agitated for 15 min. at room temperature. Subsequently, the mixture was discarded, and the microplate was washed three times with PBS, after which either 2 μg of crude venom or 5 μg of melittin/phospholipase A2 was added to each well. Polyclonal ELISA was then carried out as described by Marks *et al.* [20]. Presence of binding phages was detected using peroxidase-labelled polyclonal rabbit anti-VCSM13 antibody.

Production of monoclonal phage antibodies. Monoclonal phage antibodies were produced following the protocol devised by Tamarozzi *et al.* [19]. We used phages from the second and third rounds, separately, to infect *E. coli* TG1 in the exponential growth phase (OD of 0.4 at 600 nm). Dilutions of 10^{-10} and 10^{-11} were plated in Luria-Bertani (LB) agar medium containing 100 $\mu\text{g}/\text{ml}$ of ampicillin and 1% glucose. Isolated colonies were collected and inoculated into individual wells. For each round, two plates were prepared and agitated at 300 rpm for 12 hr at 37°C . Secondary plates were agitated at

300 rpm and 37°C until reaching an OD of 0.4 at 600 nm. The VCSM13 helper phage was added, and the plates were kept at 37°C for 30 min. After centrifugation at $1000 \times g$ for 30 min., the pellet was resuspended in LB medium supplemented with 100 $\mu\text{g}/\text{ml}$ of ampicillin and 50 $\mu\text{g}/\text{ml}$ of kanamycin, after which the plates were agitated at 300 rpm for 12 hr at 30°C . We used ELISA to determine whether supernatants containing phage antibodies specific for the crude Africanised bee venom. The phage antibodies that tested positive for venom were then tested to determine their specificity for melittin and phospholipase A2.

Production and purification of human scFv antibody fragments. Phage antibodies specific for components of the crude venom were selected for scFv production. A culture of *E. coli* HB2151 in the exponential growth phase was distributed onto a culture microplate (200 μl per well) and was infected with phage antibodies (10 μl per well), in the same positions as those employed on the primary plates. The plate was incubated for 30 min. at 37°C , after which 5 μl of transfectant was collected from each well and added to a new microplate containing 100 μl per well of LB medium supplemented with 100 $\mu\text{g}/\text{ml}$ of ampicillin and 1% glucose. The new plate was incubated at 300 rpm for 12 hr at 37°C and stored at -80°C . Selected clones were used to produce scFvs by infecting 250 ml of LB supplemented with 100 $\mu\text{g}/\text{ml}$ ampicillin and 1% glucose. The culture was then incubated at 37°C , with agitation at 300 rpm, until reaching an OD of 0.6 at 600 nm. We induced scFv expression by adding 1 mM isopropyl- β -D-thiogalactopyranoside (IPTG), and the plate was incubated for 5 hr. Extraction of periplasmic material and nickel-affinity purification by chromatography were carried out in accordance with the QIAexpressionist guide [21], which accompanies the Ni-NTA column (Qiagen, Hilden, Germany). The purification products were analysed by electrophoresis on a 12% polyacrylamide gel under non-reducing conditions. Purified scFvs were applied in a polymyxin B column (Sigma) for removing bacterial lipopolysaccharides, lyophilised and desalted on a Sephadex G-25 column (Pharmacia, Uppsala, Sweden).

Confirmation of scFv specificity for melittin and phospholipase A2. We used monoclonal ELISA for melittin and phospholipase A2 to confirm the specificity of selected clones. A glutaraldehyde-treated plate was coated with 5 μg of melittin or phospholipase A2. After sensitisation, the plate was washed three times with PBS and blocked with 2% milk-PBS for 2 hr at 37°C . The plate was again washed with PBS, and the scFv, diluted in 1% milk-PBS, was added (5 μg per well). After incubation at room temperature for 60 min., the plate was washed with PBS-Tween and PBS (three times each). Horseradish peroxidase-conjugated antipolyhistidine antibody (Sigma), diluted 1:5000 in 1% milk-PBS, was added to the plate, which was then incubated at room temperature for 60 min. After the plate had again been washed with PBS-Tween and PBS (three times each), the substrate (tetramethylbenzidine and hydrogen peroxide) was added, the reaction was stopped with 2 M sulphuric acid, and the OD was measured at 490 nm.

Kinetic haemolytic assay. Kinetic haemolytic assay was adapted from a protocol standardised in our laboratory by Tamarozzi *et al.* [19]. We used a spectrophotometer adjusted to a kinetic programme at a fixed wavelength of 700 nm. Samples were incubated at 37°C for 30 min. and analysed at 60-sec. intervals. To determine the minimum amount of venom necessary for the test, six doses were prepared (0.125, 0.15, 0.2, 0.25, 0.5 and 1.0 μg). After the minimum dose had been established, scFvs from three clones (A7, C5 and C12) were pre-incubated with the crude venom at five different scFv/venom ratios (1:1, 2:1, 3:1, 4:1 and 5:1) for 60 min. at 37°C . We then added 1 ml of an erythrocyte solution (0.05% erythrocytes) to the cuvette and performed the kinetic assay. Clone pairs were evaluated to determine whether there was a synergistic increase in inhibition.

In vivo assays. For *in vivo* tests, we determined the LD_{50} using the method devised by Karber [22].

Myotoxicity assay. Groups of four mice each were injected intramuscularly (into the gastrocnemius muscle) with $0.5 \times \text{LD}_{50}$ of bee venom pre-incubated with a combination of purified scFvs (clones A7 and C12) at an scFv/venom ratio of 3:1. Control mice received only sterile saline, scFv or $0.5 \times \text{LD}_{50}$ of crude venom. At 3 hr after the injection, plasma creatine kinase levels were measured using a commercial dosage kit (CK-Nac; Doles Reagentes, Goiânia, Brazil).

Lethality assay. Groups of four mice each were challenged intravenously (by tail vein injection) with $1.5 \times \text{LD}_{50}$ of bee venom pre-incubated with a combination of purified scFvs (clones A7 and C12), at an scFv/venom ratio of 3:1. Control mice received only sterile saline, scFv, or $1.5 \times \text{LD}_{50}$ of crude venom. Over a 36-hr period, we observed the clinical symptoms of envenomation and registered all deaths that occurred.

Statistical analysis. The statistical analysis of the myotoxicity assay results was performed with ANOVA and Duncan's *post hoc* test. Values of $p < 0.05$ were considered statistically significant. Analysis of ELISA was performed with ANOVA and Dunnett's post-test ($p < 0.05$).

Results

Phage titres.

From the first to the second round of selection, we observed a decrease in the number of eluted phages (table 1). This decrease was expected, because of the increase in the number of washes prior to phage elution. We also observed an increase in the titres of eluted phages from the second to the third round, which prompted us to conduct a fourth round of selection. The titres of precipitated phages were markedly higher than those of the eluted phages, providing evidence that the amplification was successful.

Phage antibody specificity.

Only second- and third-round precipitated phages showed relevant specificity for the crude venom (fig. 1). Eluted phage antibodies demonstrated low specificity. Increasing the number of washes contributed to the selection of more specific phage antibodies, and the third round produced more phage antibodies than did the other rounds.

Monoclonal phage antibody specificity.

Several clones isolated from the second and third rounds showed specificity for the crude venom. Despite the fact that the second-round clones showed lower specificity than did those from the third round, the number of positive clones was greater in the second round. Figure 2A illustrates the three best second-round clones in terms of specificity for the

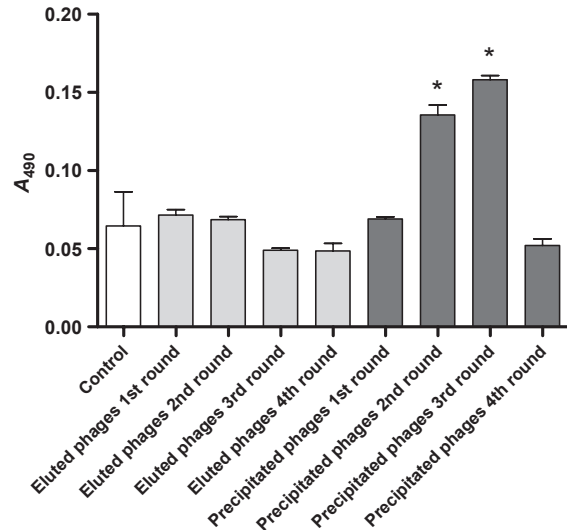


Fig. 1. Polyclonal enzyme-linked immunosorbent assay (ELISA) using eluted and precipitated phage antibodies. ELISA plate wells were sensitised with $2 \mu\text{g}$ of crude venom diluted to $100 \mu\text{l}$ with 50 mM carbonate-bicarbonate buffer, pH 9.6. Fifty microlitre of eluted or precipitated phage antibodies plus $50 \mu\text{l}$ of MPBS 1% were used. As negative control, wells were not sensitised. Presence of phage antibodies was evaluated with specific antibodies antiphage conjugated with HRP and substrate OPD/ H_2O_2 . Results were compared against the control by ANOVA and Dunnett's test ($p < 0.05$).

crude venom. Although clones A7, C5 and C12 all showed specificity for melittin (fig. 2B), only clone A7 showed specificity for phospholipase A2 (fig. 2C).

Production and purification of antibody fragments.

Production of antibody fragments was satisfactory when IPTG was used as an inducer of the lac operon. Strategy of extracting antibody fragments from the periplasm ensured better preservation of the histidine tag, thereby facilitating the purification process. Figure 3 shows the electrophoretic profiles of the purified A7, C5 and C12 clones ($1.5 \mu\text{g}/\text{well}$). The 28-kDa band represents purified scFvs. The other two bands (of approximately 15 and 60 kDa) probably represent scFv fragments and dimers, respectively.

Antibody fragment specificity.

After the ability of the A7, C5 and C12 phage antibodies to bind to melittin and purified phospholipase A2 had been verified, another ELISA was performed to determine whether the scFv molecules purified from those clones were also able to bind to the same antigens. Although all three clones were found to be specific for melittin, C5 showed lower specificity than did the others. Only clone A7 was able to bind to phospholipase A2 (data not shown).

In vitro haemolytic activity.

To make it efficient for use with Africanised bee venom, it was necessary to make some adaptations to the assay proposed by Tamarozzi *et al.* [19]. Initially, we determined the

Table 1.

Selection of phage antibodies against *Apis mellifera* venom.

Selection round	Titre	
	Eluted phages	Precipitated phages
1	8×10^6	4×10^{12}
2	2×10^5	9×10^{13}
3	4×10^6	6×10^{14}
4	7×10^5	5×10^{14}

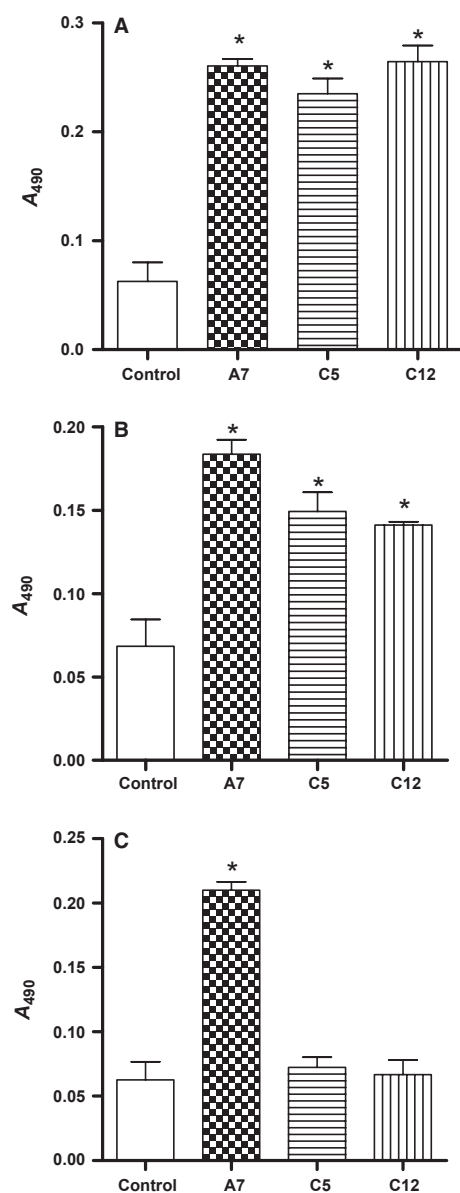


Fig. 2. Monoclonal enzyme-linked immunosorbent assay (ELISA) using phage antibodies. ELISA plate wells previously treated with glutaraldehyde were sensitised with (A) 2 µg of crude venom; (B) 5 µg of melittin or (C) 5 µg of phospholipase A2. Fifty microlitre of phage antibodies (A7, C5 and C12) plus 50 µl of MPBS 1% were used. As negative control, wells were not sensitised. Presence of phage antibodies was evaluated with specific antibodies antiphage conjugated with HRP and substrate OPD/H₂O₂. Results were compared against the control by ANOVA and Dunnett's test ($p < 0.05$).

minimum dose capable of causing 100% haemolysis. Two doses (0.5 and 0.25 µg) produced marked haemolytic activity (data not shown). The lower concentration was chosen to avoid using excessive quantities of venom and antibodies.

To test the ability of each clone in inhibiting the haemolytic activity of the venom, clones were pre-incubated with the venom individually and in pairs. Figure 4 shows the results of the kinetic haemolytic inhibition assay. The best results were obtained with clones A7 and C12 (fig. 4A,C, respec-

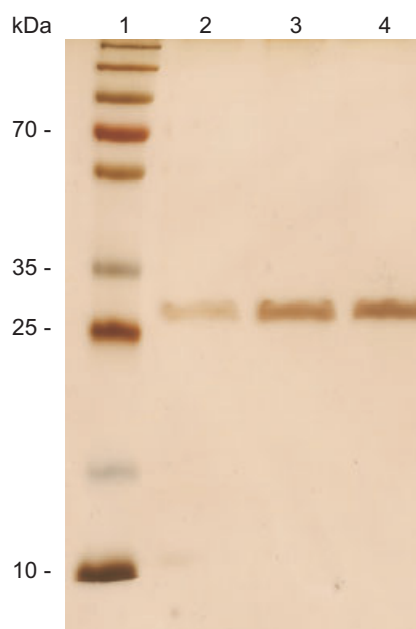


Fig. 3. Electrophoretic profile of purified A7, C5 and C12 antibody fragments. Samples of purified scFv fragments (1.5 µg) were analysed by SDS-PAGE on a 12% (w/v) polyacrylamide gel under non-reducing conditions and silver staining. Lane 1: standard molecular weight (Fermentas Life Sciences®, Burlington, ON, Canada); lane 2: scFv A7; lane 3: scFv C5; lane 4: scFv C12.

tively), which inhibited, respectively, about 95% and 80% of the haemolytic activity when incubated at the ratio of 2:1 (scFv/venom), whereas clone C5 was able to inhibit 80% only when the ratio was 4:1 (fig. 4B). Figure 5 shows the synergistic effects of clone combinations. As it can be seen in fig. 5B, the greatest inhibition was obtained with the A7–C12 clone combination at the ratio 3:1 (1.5 µg of each scFv and 1.0 µg of venom). This combination resulted in 54% haemolysis inhibition and was selected for *in vivo* assays.

Toxic effects of crude venom *in vivo*.

An LD₅₀ assay was performed to serve as a basis for the *in vivo* experiments. The estimated value was 3.9 µg/g of body-weight.

In vivo myotoxicity.

Figure 6 shows the capacity of clones A7 and C12 (combined in equal quantities) to inhibit the myotoxic effect of the crude venom. The myotoxicity of the venom was significantly lower in the group receiving crude venom pre-incubated with the A7–C12 combination than in the group receiving crude venom only ($p < 0.05$).

Lethality.

Figure 7 shows the capacity of the combination of purified A7 and C12 scFvs in delaying mice death when a lethal dose of crude venom was used. Survival was significantly higher in the mice challenged with $1.5 \times \text{LD}_{50}$ of crude venom plus the A7–C12 scFv combination than in those challenged with crude venom alone.

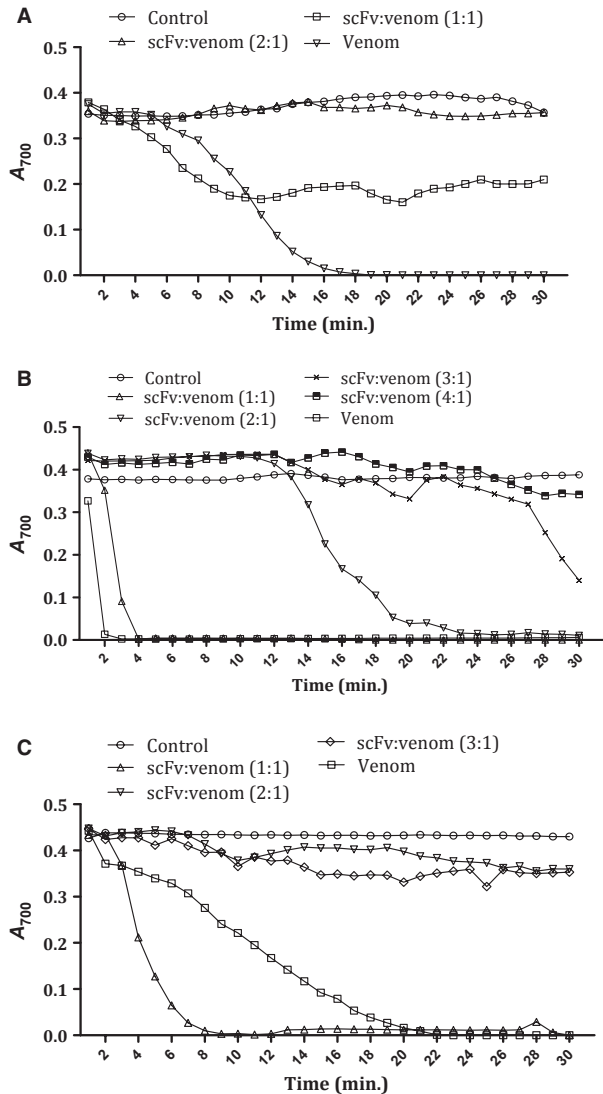


Fig. 4. Kinetic haemolytic inhibition assay using clones A7, C5 and C12 separately. Different proportions of scFv were incubated with the crude venom for 1 hr at 37°C. The reaction mixture containing 0.05% erythrocytes at 37°C was added, and the samples were measured every minute at 700 nm. (A) Clone A7; (B) clone C5 and (C) clone C12.

Discussion

There is currently no effective commercial serum for the treatment for Africanised bee envenomation. Victims of mass attacks are monitored, and their major symptoms are treated. In cases of hypersensitivity, administration of adrenaline has produced good results [23]. However, when there are a considerable number of stings, toxic reactions can overlap with anaphylactic reactions [24]. Jones *et al.* [2] produced heterologous antibodies against the venom of Africanised bees. However, that serum is not available for use in Brazil; therefore, production of antibodies by phage display technology is a potential alternative to traditional antivenom production techniques. Our group has been studying the phage

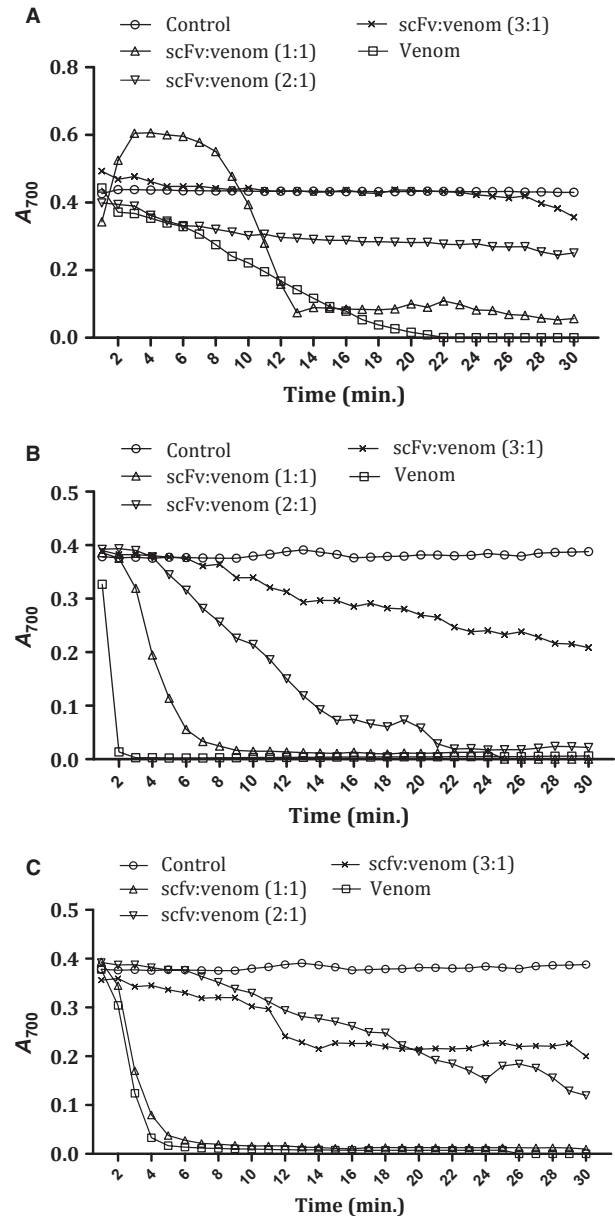


Fig. 5. Kinetic haemolytic inhibition assay using associated scFvs (A7, C5 and C12). Different proportions of scFv (1:1, 2:1 and 3:1, total scFv/venom) combinations were incubated with the crude venom for 1 hr at 37°C. The reaction mixture containing 0.05% erythrocytes at 37°C was added, and the samples were measured every minute at 700 nm. (A) A7–C5 combination; (B) A7–C12 combination and (C) C5/C12 combination.

display production of various types of antivenom (for several species) and has obtained promising results [19,25].

In the present study, the selection of binding antibody fragments specific to the crude venom was performed successfully. Three scFv clones (A7, C5 and C12) were found to bind to the crude venom and to melittin. The fact that melittin is the major component of Africanised bee venom might explain the ability of the selected phage antibodies to bind to melittin. The A7 clone was also able to bind to

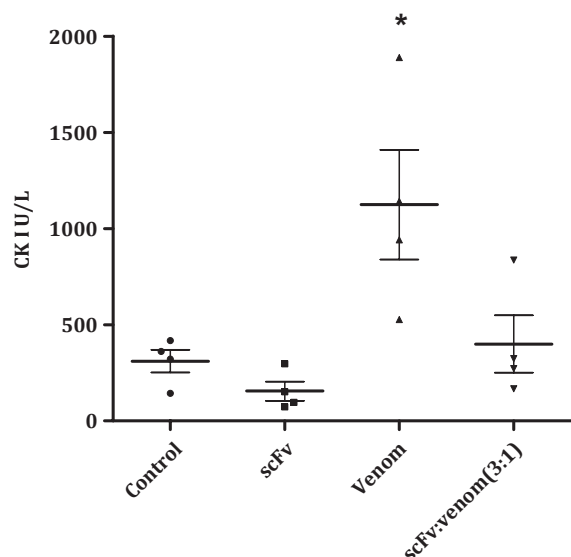


Fig. 6. Myotoxic inhibition assay using A7-C12 scFv combination. Mice were injected i.m. with saline (negative control); scFv (A7-C12) (negative control); LD₅₀ of bee venom (positive control) and ½ LD₅₀ of bee venom plus scFv (A7-C12, 3:1, total scFv/venom). After 3 hr, blood was collected and the serum creatine kinase was evaluated. Results were compared against the control by ANOVA and Duncan's test ($p < 0.05$).

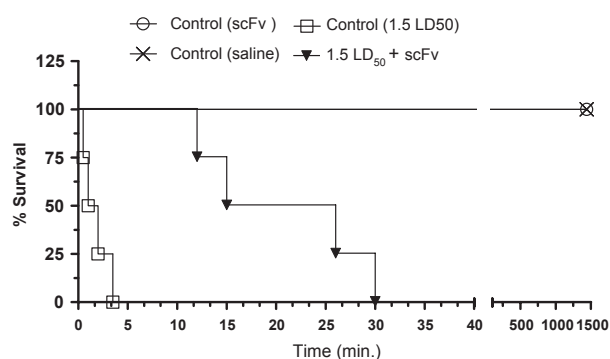


Fig. 7. Lethality inhibition assay using A7-C12 scFv combination. Mice were i.v. injected with saline (negative control); scFv A7-C12 (negative control); 1.5 LD₅₀ of bee venom (positive control) and 1.5 LD₅₀ previously incubated for 1 hr with the scFv A7-C12 combination (3:1, scFv/venom). Survival was registered during 36 hr.

phospholipase A2, which indicates that it is specific for phospholipase A2, not for melittin, and the ability of this clone in binding melittin can be attributed to the contamination of the target by phospholipase A2, information indicated by the manufacturer. According to Raghuraman and Chattopadhyay [26], presence of phospholipase A2 as a contaminant is a common problem in studies of melittin, and it can even be detected in commercial preparations.

Melittin has a random conformation and can change according to environmental conditions [27]. In addition, inactive melittin can prevent membrane pore formation [28]. Therefore, binding of scFv to melittin might have prevented

the formation of the pore and consequently the haemolysis. This effect was enhanced when the combination of two antibody fragments was used. As previously mentioned, clone C12 is specific for melittin and clone A7 is specific for phospholipase A2. Jones *et al.* [2] also developed antibodies inhibiting the phospholipase activity of crude Africanised bee venom. However, the antibody/phospholipase ratio was quite high, 400 mg/l of antibodies being required to neutralise 10 mg/l of phospholipase A2, much higher than that used in the present study.

The A7-C12 antibody fragment combination was effective in reducing the *in vivo* myotoxicity of the venom, which has been implicated in the development of acute kidney injury [29]. Several studies have demonstrated an association between bee envenomation and rhabdomyolysis, which is one of the main causes of acute kidney injury [30–32]. Therefore, inhibition of the myotoxic effects of bee envenomation can represent a strategy for preventing acute kidney injury in bee sting victims. In our laboratory, scFv fragments selected against snake venom has shown promising results in terms of reducing myotoxicity.

We also found that the A7-C12 scFv combination delayed, but did not prevent, mortality in mice receiving a lethal dose of Africanised bee venom. One possible explanation is that we employed a high dose (1.5 × LD₅₀). The fact that the antibodies were capable of inhibiting haemolytic and myotoxic activities does not imply that they are able to prevent death from bee sting, because the venom is a complex mixture of substances and provokes other symptoms, such as neurological and cardiac disorders. Various authors have reported that individuals related to bee envenomation have also presented damage at the cardiovascular system. In addition, in a canine model of Africanised bee envenomation, Walker *et al.* [33] demonstrated that the deaths were attributable to lung injury. Africanised bee envenomation can also cause loss of consciousness and death from hypoxia, hypotension and cardiac collapse [34]. Acute myocardial infarction has been reported in several case reports of patients stung by Africanised bees [35–37].

Using heterologous antibodies in an ovine model of Africanised bee envenomation, Jones *et al.* [2] observed that 20.5 mg of antivenom is required to neutralise each milligram of venom to prevent mortality. In the present study, we observed that a lower antibody/venom ratio delayed the onset of symptoms. The difficulty in reducing the lethality of other venoms has also been described by several authors. Devaux *et al.* [38] produced scFvs against the scorpion neurotoxin AahI in *Androctonus australis hector* venom and studied scFv capacity to reduce lethality in C57BL/6 mice. The authors found that mortality was prevented only when the scFv/venom ratio was approximately 3:1. Although Juarez-Gonzalez *et al.* [39] produced scFvs specific for the Cn2 toxin in *Centruroides noxius* scorpion venom, those scFvs failed to reduce the lethality of the venom.

The phage display production of two human antibody fragments (clones A7 and C12) is a promising alternative to prevent haemolytic and myotoxic effects of Africanised bee

venom. Further studies of the neutralising capacity of these two clones could lead to strategies of protection against acute kidney injury in bee sting victims.

Conclusions

The strategy proposed in the present study proved effective in selecting phage antibodies specifically against some components of the crude venom of Africanised bees. Clones A7 and C12 were able to neutralise the haemolytic activity of the venom *in vitro* and partially neutralised toxic effects *in vivo*, ensuring longer survival. Studies aiming at the production of new clones specific for other toxic components could lead to the use of these clones as components of a human serum against the venom of Africanised bees as an alternative treatment for victims of serious accidents involving bees.

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