Immunological and antimicrobial effects of venoms of endemic species of Cyprus

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Introduction/objectives/aims

Endemic species represent a great untapped resource for a variety of bioactive agents. Venoms have been reported to be a pool of antimicrobial peptides, and to have effects on haemolysis, inflammatory responses and the activation of the complement system. Only limited information exists about endemic species of venomous animals of Cyprus. We sought to study the effects of venoms from such species.

Aim: to investigate the effects of these venoms on the human complement system as well as assess their antimicrobial activity.

Methods

- The proteomic profile of the different venoms was studied using SDS-PAGE electrophoresis.
- Different concentrations of the venoms were incubated with purified C3 complement component.
- The antimicrobial activity of the venom extracts was evaluated by the spot assay method.
- Venom extracts from *Mesobuthus cyprius* (MC), *Mesobuthus gibbosus* (MG)*, *Macrovipera lebetina lebetina* (MLL) and *Vipera Ammodytes* (VA)* were tested against Gram-Positive and Gram-Negative strains.

**closely related non-endemic species used for comparison*

Results

Figure 1: SDS-PAGE of MC, MG and MLL

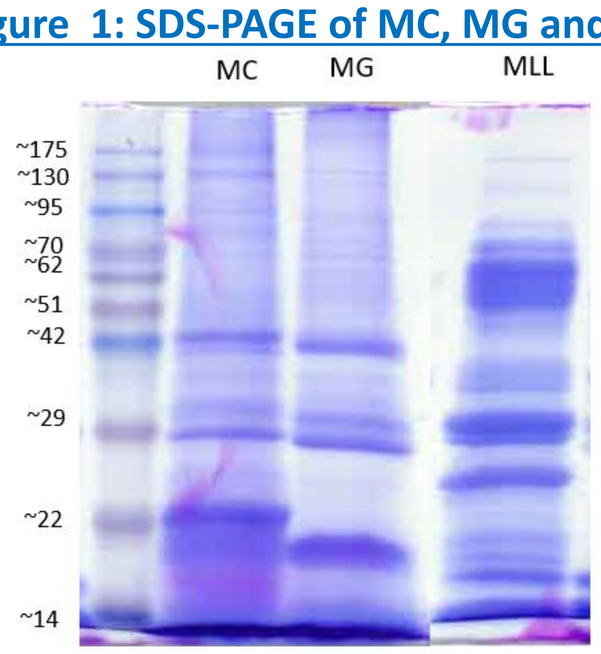
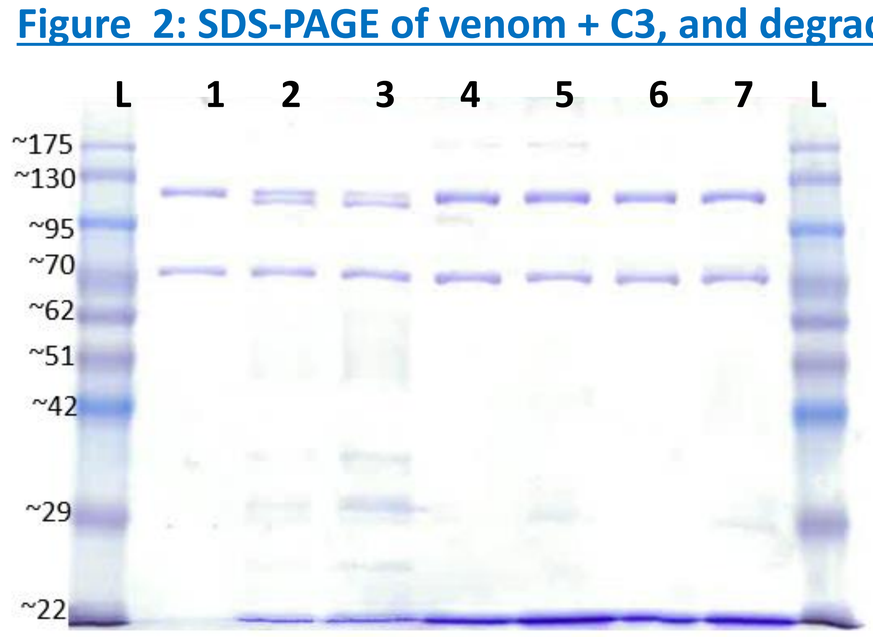


Figure 2: SDS-PAGE of venom + C3, and degradation of complement factor C3.



Samples were run under reducing conditions on 12% SDS-PAGE gels and were then subjected to Coomassie staining.
L: Ladder
1: 120 µg/mL purified C3 alone
2: C3+ 50 µg/mL MLL venom
3: C3+ 100 µg/mL MLL venom
4: C3+ 50 µg/mL MC venom
5: C3+ 100 µg/mL MC venom
6: C3+ 50 µg/mL MG venom
7: C3+ 100 µg/mL MG venom

Table 1: Antimicrobial activity of venom extracts

Bacterial Strain	Minimal inhibitory concentration[1](µg/spot)			
	Macrovipera Lebetina Lebetina	*Vipera Ammodytes*	*Mesobuthus cyprius*	*Mesobuthus gibbosus*
Escherichia coli MG1655 ATCC-700926	122	70	n/a	n/a
Staphylococcus epidermidis ATCC-14990	610	350	n/a	n/a
Streptococcus pneumoniae strain J ATCC-55143	n/a	n/a	n/a	n/a
Neisseria subflava *ATCC-49275*	n/a	n/a	n/a	n/a

[1]Minimal inhibitory concentration is defined as the concentration where total clearing of the spot occurred
n/a= no activity detected at up to 610 µg/spot of the venom extracts

Conclusion

- Different profile of proteins was seen between the different venoms showing the importance of further characterisation.
- C3 complement factor was cleaved by the venoms; most potent effect was seen with the MLL venom.
- MLL and VA venoms had antimicrobial activity against *E.coli* and *S. epidermidis*.
- We aim to expand testing to other components, parameters of complement activation and inflammation as well as other bacterial strains.

References

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