

SHORT COMMUNICATION

Characterization of Sm14 Related Components in Different Helminths by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis and Western Blotting Analysis

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Sm14 was the first fatty acid-binding protein homologue identified in helminths. Thereafter, members of the same family were identified in several helminth species, with high aminoacid sequence homology between them. In addition, immune crossprotection was also reported against Fasciola hepatica infection, in animals previously immunized with the Schistosoma mansoni vaccine candidate, r-Sm14. In the present study, data on preliminary sodium dodecyl sulphate-polyacrylamide gel electrophoresis and Western blotting analysis of nine different helminth extracts focusing the identification of Sm14 related proteins, is reported. Out of these, three extracts – Ascaris suum (males and females), Echinostoma paraensei, and Taenia saginata – presented components that comigrated with Sm14 in SDS-PAGE, and that were recognized by anti-rSm14 policlonal serum, in Western blotting tests.

Key words: Sm14 - *Schistosoma mansoni* - immune crossreaction - fatty acid-binding protein

Crossreaction among helminths has been extensively described for decades (Hillyer et al. 1988), although the true biological role of such crossreaction remained to be more clearly elucidated.

Heterologous resistance between *Schistosoma mansoni* and *Fasciola hepatica* has been reported by Hillyer and coworkers (Hillyer 1987, Christensen et al. 1987, Hillyer et al. 1988, Hillyer 1995). In previous studies it has been described that the recombinant molecule, r-Sm14, derived from *S. mansoni* adult worm extract, induces immune crossprotection against infection by *F. hepatica* in Swiss outbred mice (Tendler et al. 1996). Rodriguez-Perez et al. (1992) demonstrated a high aminoacid sequence homology between Sm14 and the *F. hepatica* fatty acid-binding protein (FABP), called Fh15, possibly responsible for the reported crossprotection.

Sm14 was the first FABP homologue identified in helminths (Moser et al. 1991). Thereafter, members of the same family were identified in several helminth species, with high aminoacid sequence homology between them: *S. japonicum*-Sj-FABP (Becker et al. 1994); *Dictyocaulus viviparus* (Britton et al. 1995); *Moniezia expansa*-*Moniezia* LBP (Janssen & Barret 1995); *Toxocara canis*-TcSL-2 (Gems et al. 1995); *Dirofilaria immitis* (Poole et al. 1996); *Echinococcus granulosus*-EgDf1 (Esteves et al. 1997); *F. gigantica*-Fg-FABP (Smooker et al. 1997); *Ascaris suum*-As-p18 (Mei et al. 1997); and *Brugia malayi*

(Michalski & Weil 1999).

The FABPs may be particularly important to schistosomes in the uptake, transport and compartmentalization of host derived fatty acids, since these parasites lack the oxygen dependent pathways required for the synthesis of sterols and fatty acids (Esteves et al. 1997).

Specifically, in the case of *A. suum*, that was included in this study, the FABP homologue component, As-p18, presented similar levels of aminoacid sequence homology, for Sm14 and Fh15 (respectively 28% and 30%) (Mei et al. 1997). Esteves et al. (1997), presented a phylogenetics analysis of helminth FABPs, in which it was demonstrated that Sm14 and Fh15 are closely related to Sj-FABP and EgDf1.

Present data refer to preliminary SDS-PAGE and Western blotting analysis of nine different helminth extracts focusing the identification of Sm14 related proteins.

Extracts were prepared basically as described previously for *S. mansoni* (Tendler & Scapin 1979, Tendler et al. 1982, Thaumaturgo et al. 2001). Additional members of the three major helminth taxonomic groups, such as *Echinostoma paraensei* (Trematode), *Hymenolepis diminuta*, *Dipylidium caninum* and *Taenia saginata* (Cestodes) and *Aspiculuris tetraptera*, *Toxocara* sp., *A. suum* (males and females) and *Toxocara canis* (Nematodes) were subjected to SDS-PAGE and Western blotting analysis (Towbin et al. 1979).

In three out of nine helminth species – *A. suum* (males and females); *E. paraensei*; and *T. saginata* – it was detected parasite proteins that clearly comigrated with r-Sm14 in SDS-PAGE. In addition, these proteins were recognized by anti-rSm14 policlonal serum, in Western blotting (Figs 1,2).

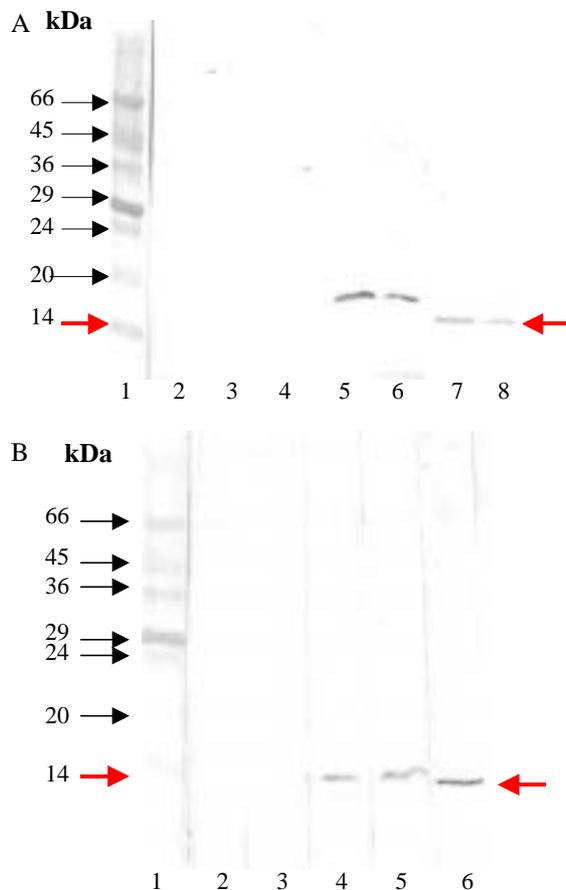
Herein presented results indicate the occurrence of immune crossreaction mediated by Sm14 and components

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Western blotting analysis of different helminth extracts against rabbit anti-rSm14 serum. A-lanes 1: low molecular weight marker, Dalton Mark VII-L Sigma; 2: *Hymenolepis diminuta* (protein concentration – 0.128 mg/ml); 3: *Aspicularis tetraoptera* (0.429 mg/ml); 4: *Toxocara* sp. (1.340 mg/ml); 5: *Ascaris suum* (males) (1.430 mg/ml); 6: *A. suum* (females) (2.060 mg/ml); 7: *Schistosoma mansoni* (males) (1.431 mg/ml); 8: *S. mansoni* (females) (1.788 mg/ml); B-lanes 1: low molecular weight marker, Dalton Mark VII-L Sigma.; 2: *Dipylidium caninum* (0.964 mg/ml); 3: *T. canis* (0.912 mg/ml); 4: *Echinostoma paraensei* (1.106 mg/ml); 5: *Taenia saginata* (1.328 mg/ml); 6: SE (1.273 mg/ml).

of different helminth species, in which homologous members to FABP family were previously detected. Considering that significant immune crossprotection has been formerly described for the related trematodes, *S. mansoni* and *F. hepatica* in animals previously vaccinated with r-Sm14, it is reasonable to further investigate this mechanism in other helminth infections.

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