MOLECULAR IDENTITY OF MAJOR CROSS-REACTIVE ADULT ANTIGENS IN FASCIOLA GIGANTICA, TOXOCARA VITULORUM AND MONIEZIA EXPANSA

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Abstract

Cross reactivity between Fasciola gigantica, Toxocara vitulorum and Moniezia expansa whole worm extracts was proved by ELISA. Intense cross-reaction was observed between F. gigantica and M. expansa rather than between each of them and T. vitulorum. As judged by immunoblot, the cross-reactive antigens in F. gigantica which recognized by T. vitulorum antisera was 109 kD while this component in addition to another one of 52 kD were detected by M. expansa sera in the same extract. Furthermore, T. vitulorum antigen which cross-reacted with F. gigantica was 133 kD and with M. expansa was 143 kD. Antigens responsible for cross-reactivity in M. expansa were 130 kD and 210 kD to T. vitulorum and F. gigantica respectively.

Introduction

Cross-reaction between nematodes was previously reviewed. Some of the earlier studies focused on the detection of significant homology in the total protein make-up of crude extracts (Charely et al., 1981; Milner et al., 1987 and Hayat et al., 1997). While other attempts concerned with the identification and isolation of certain molecules which are responsible for this cross-reactivity. For instance, a molecule of 55-66 kD appeared to be a major cross-reactive antigen between Toxocara canis and Ascaris suum as reported by Nunes et al. (1997). Meanwhile biotinyl enzymes in nematodes were proved to be
an important source of cross-reactivity among *T. canis*, *A. suum*, *Trichinella spiralis* and *Trichuris muris* (Lorenzo et al., 1999). Cross-reaction was also observed between trematode species. Where comparative immunological studies of *Fasciola hepatica* and *Schistosoma mansoni* were the best explanation of this phenomenon (Hillyer, 1995 and Shousha et al., 1999). On the other hand, studies related to cestodes cross-reaction were relatively little. A homology of the C-terminal region of neuropeptides of *Proteocephalus pollanicola* and *Moniezia expansa* was suggested to be responsible for cross-reactivity between both cestodes (Marks et al., 1993). Cross-reaction was not only restricted to species belong to the same phylum but also extended to helminths of different phyla (Sakolvaree et al., 1997; Konishi, 1997 and Dekumyoy et al., 1998). Although the existence of cross-reactive antigens is a major cause of inaccurate diagnosis, they could be used for cross-protection against parasitic infections (Hillyer, 1985 and 1995).

In the present study, work aimed to investigate the possible immunological cross-reactivity between *Fasciola gigantica* (*F. gigantica*), *Toxocara vitulorum* (*T. vitulorum*) and *Moniezia expansa* (*M. expansa*). And to identify, at the molecular level, both species-specific and cross-reactive antigens which could be of significance as vaccine candidates.

**Materials and Methods**

**Preparation of whole worm antigens.** *F. gigantica*, *T. vitulorum* and *M. expansa* were collected from animals slaughtered at Cairo abattoir. Mature worms were washed thoroughly with distilled water to remove all traces of host tissues and bile. Antigens were prepared by homogenizing helminths, separately, in 0.15 M PBS, pH 7.5 supplemented with 2 mM phenyl methyl sulphonyl fluoride (PMSF) and 0.02% NaN₃ in a Ten Broeck tissue grinder. The homogenates were sonicated and then centrifuged at 18,000 rpm at 4°C for 1 h. Clear supernatants corresponding to each worm preparation were separately pooled to give the *F. gigantica* antigen (FgA), *T. vitulorum* antigen (TvA) and *M. expansa* antigen (MeA). The protein concentration in each antigen was determined by the method of Lowry et al. (1951).

**Rabbits' immunization with whole worm antigens.** Six rabbits (2 per species) were immunized subcutaneously with 20 mg of either FgA, TvA or MeA in Freund's complete adjuvant. A booster dose of 20 mg antigen in Freund's incomplete adjuvant was injected on day 14. Second and third booster doses were given on day 21 and 28.
respectively and serum samples were collected 4 days after the last immunization. Rabbit anti FgA (RAFgA), anti TvA (RATvA) and anti MeA (RAMeA) were evaluated by ELISA using antigen of respective parasite, aliquoted and stored at -20°C until use.

**Enzymes-linked immunosorbent assay (ELISA).** The assay was performed as described by Santiago et al. (1986) with slight modifications. ELISA OD values cut off were calculated according to Allan et al. (1992).

**Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE).** Extracts of the three helminths were boiled separately in sample buffer containing 5% 2-mercaptoethanol for 3 min, centrifuged and supernatants were electrophoresed on SDS-polyacrylamide slab gels according to procedures described by Laemmli (1970).

**Immunoblotting.** Protein bands were electrophoretically transferred from SDS-polyacrylamide gel to nitrocellulose sheets according to Towbin et al. (1979) in a mini blotting system at 10 v/gel overnight. The sheets were cut into strips and incubated with the three prepared diluted antisera. After washing, the strips were incubated with IgG anti-rabbit alkaline phosphatase. The strips were exposed to substrate solution for 30 min then rinsed thoroughly with distilled water to stop the reaction.

**Results**

Analysis of antibody binding activities in RAFgA, RATvA and RAMeA. (A) Specific binding activities: The three figures (1,2, & 3) showed comparable strong binding patterns with serially diluted antisera with a common trait of high activity binding of antigens with high sera concentrations (OD values ranged from 1-0.975). High dilution of antisera (1:4096) still reacts with its respective antigens to give OD values of 0.616 with FgA, 0.654 with TvA and 0.779 with MeA (Fig 1, 2 and 3 respectively). (B) Cross-reactive binding activities: Results depicted in the three figures proved the presence of anti cross reactive antigenic determinants in the three antisera. Reactivity of RAFgA towards MeA is stronger than that with TvA. Cross-binding capacity of RATvA towards MeA is slightly higher than towards FgA. Where the cross binding activities were very close at different dilution of both antisera particularly at 1:2 and 1:128. RAMeA showed higher activity binding with FgA than with TvA. Using rabbits hyperimmune sera in immunoblot, it was possible to identify both species-specific and cross-reactive antigens in the three extracts. (A) Species-specific antigens. RAFgA detected 4 bands of
molecular weights 225, 109, 74 and 24 in FgA (Fig 4 lane A). While RATvA recognized 5 specific polypeptides in TvA of 227 kD, 143 kD, 133 kD, 88 kD and 47 kD (Fig 5 lane A). It was also observed that specific components of MeA in the region of 236 kD, 210 kD, 130 kD, 50 kD and 35 kD were identified with homologous antisera, RAMeA, (Fig 6 lane A). (B) Cross-reactive antigens. The cross-reactive antigens in FgA, which were recognized by RAMeA, were 109 kD and 52 kD (Fig 4 lane B). Whereas the component of 109 kD was only recognized by RATvA in the same extract (Fig 4 lane C). Meanwhile, a polypeptide of 143 kD in TvA was responsible for cross-reactivity with M. expansa (Fig 5 lane B) while a component of 133 kD was responsible for cross-reactivity with F. gigantica (Fig 5 lane C). Moreover, M. expansa antigens, which cross-reacted with FgA and TvA were 210 kD and 130 kD respectively (Fig 6 lane B and C).

![Graph](image)

**Fig. 1:** The binding capacity of RAFgA towards FgA, TvA and MeA.
Fig. 4 Comparative immunoblot of *F. gigantica* polypeptides recognized by various antisera: RAFgA (A), RAMeA (B) and RATvA (C).

Fig. 5 Comparative immunoblot of *T. vitulorum* polypeptides recognized by various antisera: RATvA (A), RAMeA (B) and RAFgA (C).

Fig. 6 Comparative immunoblot of *M. expansa* polypeptides recognized by various antisera: RAMeA (A), RAFgA (B) and RATvA (C).
Discussion

In the present study we proved cross-reaction between helminths that were not previously known to be cross-react. The selection of these helminths was based on their economic and medical veterinary importance and also to prove that immunological cross-reactivity takes place regardless the biology, the structure and the behaviour of helminths in their hosts.

As inferred from ELISA results, a hyperimmune serum raised against one extract cross reacts with the other extracts. However, OD values obtained in homologous assays are higher than those in heterologous ones. These results are in accordance with that of Smith et al. (1983) who observed high reactivity of ascarid antigens with their respective antisera. Conversely, Cuéllar et al. (1995) observed an intense cross-reaction between Toxascaris leonina, T. canis and A. suum where heterologous assays recorded high values than homologous ones. Discrepancies between these observations may either be due to differences in antigenic determinants utilized or assays used.

Cross-reactions elucidated in the present study between the three helminths were observed fundamentally at low sera dilutions. But clear distinction between specific and cross-reactive binding activities was clearly observed with highly diluted sera. This observation was particularly noticed with RAFgA and RAMeA towards heterologous antigens while RATvA showed higher specific binding activities than cross-reactive ones at any sera dilution. Based on these observations, cross-reactions between these helminths could be avoided or at least minimized by diluting the examined sera prior investigations.

RAFgA contains high cross-reactive binding activities to MeA than towards TvA. This was also confirmed by intense cross-reaction between RAMeA and FgA. In earlier attempt, cross-reaction between FMRFamide-related peptide (FaRP) in F. hepatica and neuropeptide F(NPF) in M. expansa was exhibited with immunochemical techniques (Marks et al., 1995). In contrary, Larramendy and Pedroso (1984) proved that there was no significant cross reactivity between F. hepatica and M. expansa as assessed by double diffusion, immunoelectrophoresis and immunofluorescence. This conflict can be explained by the fact that cross-reaction between helminths depends on the type of antigen (Poot et al., 1997; Ikeda, 1998) and the technique utilized (Alarcon de-Noya et al., 1997).

The proved cross-reactivity between F. gigantica, T. vitulorum and M. expansa as revealed by ELISA in the present study encouraged interest on the molecular nature of antigens responsible for this cross-
reactivity. As revealed by immunoblotting assay, the cross-binding activities in RAFgA were directed towards a component of 133 kD in TvA and a component of 210 kD in MeA. While RATvA recognized one component in FgA of molecular weight 109 and identified another component of 130 kD in MeA. RAMeA detected a polypeptide of 143 kD in TvA but recognized 2 polypeptides of 109 kD and 52 kD in FgA. Of interest was a component of 109 kD which was detected by both RATvA and RAMeA in *F. gigantica*. Consequently, this component is common between the three helminths. Moreover, the same component was also detected by RAFgA, which proved that a single immunogenic molecule might express both cross-reactive and highly specific epitopes (Aronstein et al., 1986). This phenomena was also observed with cross-reactive antigens in both TvA (143 kD and 133 kD) and MeA (210 kD and 130 kD) which were also detected by their respective sera. Meanwhile, a polypeptide of 52 kD in FgA which was detected by RAMeA was not recognized by its homologous antisera which indicates that this molecule is responsible only for cross-reactivity. Based on this observation, it is tempting to speculate that intense cross-reaction observed between FgA and MeA is highly attributed to this antigen.

In conclusion, the current study established the cross-reaction between three helminths belong to different phyla, in addition to identification of two sets of polypeptides which are species-specific and cross-reactive. The proved cross-reactivity between the three helminths suggests that a vaccine developed against one helminth infection could also give effective protection against heterologous species. Of relevance to this assumption is the observation of Hillyer (1985 and 1995) who demonstrated that *F. hepatica* antigens which cross-reacted with *S. mansoni* conferred protection against challenge infection with *S. mansoni* cercariae in mice. While non cross-reactive antigens did not exhibit that protection. Further investigations will be essential to isolate the cross-reactive antigens and to evaluate their potency in protection against heterologous infections.

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