Significance of a common 65 kDa antigen in the experimental fasciolosis and toxoplasmosis

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Abstract In the current study, cross-reaction between two important zoonotic parasites; extracellular helminthes *Fasciola gigantica* and intracellular protozoa *Toxoplasma gondii* was proved by enzyme linked immunosorbent assay. Five antigens were used to identify and compare the cross-binding activities in the prepared antisera. Two *F. gigantica* antigens; adult flukes (FgA) and eggs (FgEA) were used to detect IgG in *T. gondii* naturally infected human sera (TgIHS) and experimentally infected sera of sheep (TgISS), mice (TgIMS) and rats (TgIRS). Three types of *T. gondii* antigens; RH (TgRHA), local sheep isolate (TgLA) and ME49 isolate (TgMEA) were used to detect cross binding activities in *F. gigantica* experimental infected rabbit sera (FgIRS) and *F. gigantica* naturally infected bovine sera (FgIBS). The cross-binding activities in the prepared antisera were strongly directed towards FgA and TgLA rather than the other antigens. The characterization of the five antigens using SDS-PAGE showed 4 common bands of FgA and TgLA; 165, 97, 76, and 65 kDa. While two common bands were observed between TgRHA, TgMEA and FgA; 165, and 65 kDa. Whereas, two common bands found between three types of *T. gondii* antigens and FgEA were identified; 165 and 65 kDa. The immunogenic cross-reactive bands between FgA and TgLA with *F. gigantica* infected bovine sera were identified by immunoblot. In FgA, the common immunogenic bands

were 165, 65 and 14 kDa. While in TgLA, common immunogenic bands were 165 and 65 kDa. Whereas, the common immunogenic band between FgA and TgLA identified with *T. gondii* experimentally infected sheep sera was 65 kDa. The current research proves cross reaction between *F. gigantica* and *T. gondii*. One common band of 65 kDa showed broad immunogenic cross-reactivity with the developed antisera raising the prospect of being putative common immunodiagnostic candidate of both infections.

Keywords Cross reactive antigens · *F. gigantica* · *T. gondii* · ELISA · SDS-PAGE · Western blot

Introduction

The sharing of molecules among organisms is an expected finding because there are many molecules, such as enzymes, hormones, receptors, etc., that have been conserved during evolution. The sharing of molecules able to elicit immune responses between different species of various genera is known as antigenic community, and it is responsible for antigenic cross-reactivity (Losada et al. 2005). This has special relevance for the identification of molecules with potential for drug or vaccine development effective against different species or genera of organisms.

One of the famous models of helminthes cross reactivity is that observed between *Fasciola hepatica* and *Schistosoma mansoni*. Fatty acid-binding proteins (FABP-Fh15 and Sm14) are among the characterized common proteins between both worms and exploited as a potential dual-vaccine (Ramajo et al. 2001; Vilar et al. 2003; Ramos et al. 2009). Recently, a proteomic-based approach identified 28 immunoreactive proteins that are common to both adult

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F. hepatica and *S. mansoni*. Some of the identified proteins could be used to develop vaccines against fasciolosis and schistosomiasis (Boukli et al. 2011).

Concerning *Toxoplasma*, the apical membrane antigen 1 is a cross-reactive antigen between *Neospora caninum* and *Toxoplasma gondii* and the antibodies directed toward this antigen from *N. caninum* inhibits host cell invasion by both parasites (Zhang et al. 2007). Additionally, a strong cross reaction between *Trichinella spiralis* muscle larval antigens and *T. gondii* tachyzoites was recorded with four common immunoreactive proteins (Hassanain et al. 2009). Moreover, the potent immune responses to *T. gondii* were capable of suppressing the responses to *F. hepatica* infection (Miller et al. 2009). The current research is an attempt to prove cross-reaction between *F. gigantica* and *T. gondii* and to identify common components and evaluating the possibility of utilizing these components as diagnostic antigens for both infections.

Materials and methods

Antigen preparation

Fasciola gigantica adult fluke and egg antigens

Live adult flukes and eggs were homogenized separately in 0.1 M phosphate buffer saline (PBS) pH 7.2 as method described by Oldham (1983). The homogenates were then subjected to ultra-sonication at maximum amplitude (peak to peak) for 10 min, two times with an interval of 1 min using an ultra sonicator (Misonix, USA) and centrifuged at 18,000 rpm for 45 min at 4 °C. The supernatants were collected and designated as *F. gigantica* (FgA) and crude *F. gigantica* egg antigen (FgEA).

Toxoplasma gondii antigens

Virulent (RH), locally isolated and ME isolates of *T. gondii* were maintained in Department of Zoonosis, National Research Center, by serial passage in mice according to the method of Johnson et al. (1979). *T. gondii* tachyzoites antigens of the three isolates were prepared as described by Waltman et al. (1984). Briefly, tachyzoites were repeatedly freeze and thawed to rupture the parasite wall, sonicated, centrifuged at 18,000 rpm for 45 min at 4 °C and the supernatants were collected.

Determination of antigens protein content

The prepared antigens were separately assayed for its protein content by the method of Lowry et al. (1951), then aliquot and kept at −20 °C until use.

Experimental infection of rabbits with *F. gigantica*

Six rabbits were infected orally with viable *F. gigantica* encysted metacercariae, 30 metacercariae for each (Muro et al. 1997). Blood samples were taken from ear vein at 2 weeks intervals starting 15 days post infection until 60 days. Serum samples were stored at −20 °C for serological analysis and designated FgIRS.

Fasciola gigantica naturally infected bovine sera

Blood samples of heavily parasitologically infected buffaloes with *F. gigantica* were collected and sera were isolated as positive reference bovine sera (FgIBS).

Experimental infection of sheep, mice and rats with *T. gondii* RH isolate

Two 18-month-old sheep, 20 mice and 10 rats were experimentally infected with RH strain of *T. gondii* as the procedures described by Dubey and Beattie (1988). All animals were proved sero-negative for *T. gondii*-specific IgG using modified agglutination test (MAT) before experimental infection. Sera were collected 2 months post infection, kept at −20 °C for serological analysis and designated TgISS, TgIMS and TgIRS respectively.

Toxoplasma gondii naturally infected human sera

Toxoplasma gondii strongly positive human sera, confirmed by MAT at 1:25 dilution as a cut-off point, were chosen as positive reference and designated TgIHS.

Enzyme linked immunosorbent assay (ELISA)

The assay was adopted to detect cross binding activities in TgISS, TgIMS, TgIRS and TgIHS with *T. gondii* toward FgA and FgEA. Moreover, cross binding activities in sera obtained from experimentally infected rabbits and naturally infected buffaloes with *F. gigantica* toward the three *T. gondii* isolates tachyzoite antigens; RH (TgRHA), local (TgLA) and ME 49 (TgMEA) were also detected by ELISA. The optimum antigens concentrations and conjugates dilution were determined by checkerboard titration where two fold serially diluted antibodies were used. The test procedures were carried out according to the method described by Lind et al. (1997) with little modifications. The cut off values of optical densities (OD) were calculated according to Hillyer et al. (1992).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Proteins of FgA, FgEA, TgRHA, TgLA and TgMEA were separately electrophoresed on SDS-PAGE according to the

method of Laemmli (1970). After separation, the gel was fixed in 50 % methanol and stained with Silver stain according to the method of Wray et al. (1981). Molecular weight standards were electrophoresed on the same gel to calculate the relative molecular weights of the examined antigens.

Western blot

Immunoblot assay was utilized to identify the cross-reactive components recognized in FgA and TgLA by both TgISS and FgIBS. The assay was carried out as the method described by Towbin et al. (1979).

Results

Cross binding activities in *T. gondii* infected serum samples to *F. gigantica* antigens

Figures 1 and 2 proved cross-reaction between *Fasciola* antigens and *T. gondii* infected sera. The profiles of cross reactivities differ based on the used antigen; FgA or FgEA. The cross reactivities in TgIHS to FgA are higher than to FgEA. While cross binding capabilities in TgISS to FgEA is higher than to FgA.

Cross binding activities in *F. gigantica* infected serum samples to *T. gondii* antigens

Cross-reaction between *F. gigantica* infected sera and *T. gondii* antigens was proved in Fig. 3. The cross reactivities in FgIRS 15 and 30 days post infection are higher

Fig. 1 Cross-reactive binding activities in TgISS, TgIHS, TgIMS and TgIRS toward FgA

Fig. 2 Cross-reactive binding activities in TgISS, TgIHS, TgIMS and TgIRS toward FgEA

Fig. 3 The cross-reactive binding activities in FgIRS 15, 30, 45 and 60 days post infection toward TgRHA

than in FgIRS 45 and 60 days post infection and FgIBS to TgRHA. Moreover, cross binding capabilities in FgIBS are lower than that in experimentally infected rabbit sera to TgLA (Fig. 4) and to TgME (Fig. 5). Cross binding activities in experimentally infected rabbit sera to TgLA are higher than that to TgRH and TgME (Figs. 3, 4, 5).

Electrophoretic profile of the five antigens

Figure 6 (lanes 2–6) demonstrated complex separation pattern of antigens and also showed extensive electrophoretic similarity between the five antigens especially in terms of 165 and 65 kDa. Nevertheless exclusive molecules to *Fasciola* antigens were detected as 55 kDa

Fig. 4 The cross-reactive binding activities in FgIRS 15, 30, 45 and 60 days post infection toward TgLA

Fig. 5 The cross-reactive binding activities in FgIRS 15, 30, 45 and 60 days post infection toward TgMEA

selectively expressed by FgA and FgEA (Fig. 6, lanes 2, 3) and 53 kDa and 29 kDa associated only with TgLA (Fig. 6, lane 4). 80, 47 and 30 kDa were detected only in TgRHA (Fig. 6, lane 5) while 45 and 24 kDa were associated only with TgMEA (Fig. 6, lane 6). Also there are common bands between FgA and TgLA; 165, 105, 97, 76 and 65 kDa (Fig. 6, lanes 2, 4), respectively.

Cross-reactive immunogenic components between *F. gigantica* and *T. gondii*

Immunogenic cross-reactive components in TgLA that recognized by FgIBS were 165 and 65 kDa as proved by

Fig. 6 Comparative SDS-PAGE pattern of five antigens. lane 2 FgA; lane 3 FgEA; lane 4 TgLA; lane 5 TgRHA; lane 6 TgMEA and lane 1 molecular weight standards in kDa

immunoblot (Fig. 7, lane 3). Furthermore, TgISS identified one cross-reactive band in FgA of molecular weight 65 kDa (Fig. 8, lanes 3, 4).

Discussion

The current study proved cross-reaction between two economically important zoonotic parasites belong to extracellular helminthes and intracellular protozoa. This cross-reaction was fundamentally observed by ELISA in which antisera raised against one antigen cross-reacted with the other antigen. The selection of *Fasciola* stages depends on worm representing adult stage and eggs representing the embryonic stages and the use of ELISA in the present research was based on previous studies suggested it as a good tool for epidemiological surveys of toxoplasmosis (Gamble et al. 2005) and fasciolosis (Anuracpreeda et al. 2013).

The current result considered additional evidence of widely spread phenomenon of cross reaction among parasites (Abdel-Rahman and Abdel-Megeed 2000; Espindola

Fig. 7 Identification of antigenic target recognized by FgIBS in FgA, lane 2; in TgLA, lane 3 and in FgEA, lane 4. Molecular weight standards in kDa are demonstrated in lane 1

et al. 2000; Pardini et al. 2002; Abdel-Rahman et al. 2003; Ishida et al. 2003; Ferrer et al. 2003; Noeli et al. 2005; Kandil et al. 2005; Hassanain et al. 2009; El-Moghazy and Abdel-Rahman 2012). Cross binding activities in experimentally infected rabbit sera with *F. gigantea* to TgLA are higher than to other two types; TgRH and TgMEA. Moreover, the cross binding activities in FgIBS toward the three antigens of *T. gondii* are lower than that in FgIRS which may be due to higher titer of antibodies in experimentally infected rabbit serum than naturally infected bovine serum. Furthermore, higher OD values were recorded to TgLA when reacted with FgIBS than the other two isolates and this is probably attributed to the high potentials of TgLA in detecting cross-reactive antibodies to

Fig. 8 Identification of antigenic target recognized by TgISS in FgA, lane 2; in TgLA, lane 3 and FgEA, lane 4. Molecular weight standards in kDa are demonstrated in lane 1

F. gigantea. This observation is probably due to the presence of more common components in TgLA with FgA than other antigens. Moreover, TgLA is a virulent Egyptian mutton isolates (Shaapan and Khalil 2008; Hassanain et al. 2011; Dubey et al. 2012) while, RH strain and ME49 are isolates significantly more associated with human congenital toxoplasmosis than with animal infection (Howe and Sibley 1995).

The variable complex electrophoretic profiles of the three antigens of *Toxoplasma*, in the current study, reflect the different cross-reactive potentials of them. Where, the antigen TgLA consists of 165, 116, 97, 90, 76, 70, 65, 55 and 27 kDa, TgRH antigen consists of 165, 146, 116, 90, 80, 65, 47 and 30 kDa and TgME antigen composed of 165, 97, 90, 65, 45 and 24 kDa. On the other hand, the demonstrated differences in antigenic profiles suggest that western blot is a valuable test for differentiation of

Toxoplasma strains (Delibas et al. 2006). Common bands between the two antigens FgA and TgLA are 165, 97, 76 and 65 kDa. While, those common between FgA, TgRH and TgME are 165 and 65 kDa. These bands are possibly responsible for the proved cross-reaction. But, the hypothesis must be proved by immunoblot to identify which of the common bands are immunogenic. A common 65 kDa was identified in FgA and TgLA by both FgIBS and TgISS. This finding proves its significance in the diagnosis of both fasciolosis and toxoplasmosis than 165 kDa that exists in both antigens, identified by FgIBS but did not identify by TgISS.

In immunoblot, infected buffalo serum was utilized because buffalo is a natural host and from which both worms and eggs were collected while *Toxoplasma* infected sheep serum was selected because locally isolated *Toxoplasma* antigen is of sheep origin. A 65 kDa protein is one of the famous diagnostic antigens of animal toxoplasmosis. Importantly, *T. gondii* matrix antigen 1 (TgMAG1), of 65 kDa protein, which is abundantly expressed in both bradyzoites and tachyzoites, was evaluated as a candidate for the development of a diagnostic reagent for ovine toxoplasmosis (Tumurjav et al. 2010). A 65 kDa fraction isolated from *T. gondii* tachyzoites of horse origin was successfully utilized in the diagnosis of human toxoplasmosis (Hassan et al. 2012). Moreover, a 65 kDa included in affinity isolated fraction of the local camel strain tachyzoites was successfully utilized in the diagnosis of cattle toxoplasmosis (Toaleb et al. 2013).

The new finding in the current research is the identification of 65 kDa in *Fasciola* adult worm and egg by *Toxoplasma* infected sheep serum using immunoblot. Moreover the isolation of this antigen from *Fasciola* worm and evaluating its diagnostic potency of toxoplasmosis merit further research.

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