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Magnetic affinity enzyme-linked immunoassay based on recombinant 26 kDa glutathione-S-transferase for serological diagnosis of schistosomiasis japonica

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ABSTRACT

Schistosomiasis remains a serious worldwide public health problem. Improving the diagnostic assay for surveillance and monitoring will contribute to hastening the possible elimination of the disease in endemic regions. Therefore, this study aims to develop magnetic affinity enzyme-linked immunoassay (MEIA) for serological diagnosis of schistosomiasis based on recombinant 26 kDa glutathione-S-transferase of *Schistosoma japonicum* (rSj26GST). BALB/c mice infected with *S. japonicum* cercariae (40 per mouse) were used. After infecting for 6 weeks, the antibody was detected by MEIA. All of the infected mouse sera were effectively determined by MEIA. Compared with the enzyme-linked immunosorbent assay (ELISA), MEIA has a higher ratio of the mean positive value to the mean negative value (P/N) at the same dilution ratio (3.92 versus 2.66). MEIA was further applied for diagnosis of human schistosomiasis. Sera from 28 schistosomiasis-confirmed patients with low-intensity infection, 15 treated patients, and 20 non-endemic negative controls, were used to assess the assay. The results showed that MEIA and ELISA had similarity in positive detection rates. However, the higher P/N of MEIA was observed at the same dilution ratio. MEIA had high negative rate in detection of specific IgG in the treated patients. Moreover, there was no cross reaction with the sera of paragonimiasis patients. These results suggested that MEIA based on rSj26GST is a simple, rapid, convenient assay for the diagnosis of schistosomiasis.

1. Introduction

Schistosomiasis is a tropical parasitic disease caused by blood-dwelling fluke worms of the genus *Schistosoma* (Gryseels et al., 2006). In the past half century, tremendous success in control of *Schistosoma japonicum* has been achieved in China, however, schistosomiasis is not completely controlled, and the transmission continues in many regions (Zhou et al., 2005).

The current epidemiological situation with low prevalence and low intensity of infections has decreased the diagnostic value of commonly used tests. A definite diagnosis can be confirmed by parasitological techniques, including miracidium hatching test and Kato-Katz thick smear. However, these methods are time-consuming and limited in sensitivity (Engels et al., 1996; Yu et al., 1998; Utzinger et al., 2001). Immunodiagnostic methods, especially circumoval precipitin test (COPT), indirect hemagglutination assay (IHA), enzyme-linked immunosorbent assay (ELISA) and dipstick dye immunoassay (DDIA), are widespread in endemic areas (Zhu, 2005). At present, these immunodiagnostic assays for schistosomiasis detection are relatively time-consuming and require well-trained personnel and special instruments so as to limit their use in the detection fields (Yu et al., 2011). It has been reported that assays based on polymerase chain reaction (PCR) techniques are capable of detecting deoxyribonucleic acid (DNA) released from schistosome (Pontes et al., 2003; Lier et al., 2008). More recently, loop-mediated isothermal amplification (LAMP) is a simple, rapid and sensitive technique. The LAMP assay has already been used for amplification of DNA of other microorganisms including parasites, and notably *S. japonicum* (Thekisoe et al., 2007; Xu et al., 2010; Wang et al., 2011). At present, practical use of LAMP in field tests for diagnosis of schistosomiasis and for monitoring schistosomiasis transmission still require further system development and validation. Therefore, there is an urgent need for a simple, rapid, sensitive, reliable and low cost assay to screen schistosomiasis patients on a large scale.

Magnetic beads have been widely used as carriers of antibodies for immunoassay, cell separation and tissue typing (Olsvik et al., 1994). In addition, magnetic beads have also been applied for schistosomiasis diagnosis (Teixeira et al., 2007; Lei et al., 2009). The main advantages of the magnetic bead-based immunoassay include: increasing the surface area for immobilization of antigen or antibody, reducing the incubation time, increasing sensitivity and making easy manipulation (Hayat et al., 2011). In our

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previous work, we have developed magnetic bead-based immunoassay against soluble egg antigen (SEA) for *S. japonicum* antibody detection (Liu et al., 2010). However, there is a cross-activity problem when using crude extracts as diagnostic antigens, which contain many antigens that might be shared with unrelated pathogens (Tsang and Wilkins, 1997; Maddison, 1991). For this reason, a defined diagnostic antigen, which might increase sensitivity and specificity of a serological assay, is required.

The use of purified and recombinant antigens might provide a better effect than the use of crude extracts, and provide additional information related to duration of infection, distinctive pathology or protective immunity (Doenhoff et al., 2004). Currently, there are already some recombinant antigens as diagnostic antigens available, e.g., 26-kDa glutathione-S-transferase (GST), fatty acid binding protein (FABP), signaling protein 14-3-3, 97 kDa paramyosin, and 22-kDa tegumental membrane-associated antigen (TEG) (Jiz et al., 2008; Wei et al., 2010; Qian et al., 2011; Li et al., 1999; Luo et al., 2009).

In this work, rSj26GST was used for diagnosis of schistosomiasis japonica with magnetic affinity enzyme-linked immunoassay (MEIA) and comparison was made with the ELISA method which is currently used in China.

2. Materials and methods

2.1. Strains and plasmids

The recombinant vector pET28a containing coding sequence of Sj26GST, was transformed in *Escherichia coli* BL21 (DE3). Strain of *E. coli* BL21 (DE3) and expression vector pET28a were stored in our laboratory.

2.2. Animal serum samples

Eight BALB/c mice (male, 6 weeks) were provided by the Experimental Animal Facility of Tongji Medical College, China. Each anesthetized mouse was infected percutaneously with 40 *S. japonicum* cercariae. The sera were collected before infection and after infection for 6 weeks. All sera were stored at −20 °C until use. Animal care and use were performed in compliance with the "Regulations for the Administration of Affairs Concerning Experimental Animals in Hubei Province" prepared by the China Hubei Provincial Science and Technology Department in 2005.

2.3. Human serum samples

Sera were obtained from 28 individuals with low-intensity infection of *S. japonicum*, wherein the egg count of each individual is less than 100 eggs per gram (<100 epg) in the feces. All the patients were from the endemic regions where they received repeated praziquantel chemotherapy. 15 sera were collected at the post-treatment (6 months after chemotherapy) stage and confirmed negative through Kato-Katz method. Control sera were collected from 20 healthy adults from non-endemic area. Six sera infected with paragonimiasis confirmed parasitologically, serologically diagnosed were assayed and they did not present *S. japonicum* eggs in feces.

2.4. Expression and purification of rSj26GST

The fusion proteins were expressed in *E. coli* BL21 (DE3), purified by affinity of nichel nitrilotriacetic resin with His6-tag, and eluted in an imidazole gradient. Imidazole was removed by using PD-10 desalting column (Pharmacia, Sigma) by AKTA purifier (GE, USA). The recombinant antigens were collected and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Protein concentration was determined by BCA protein assay.

2.5. Coupling rSj26GST to magnetic beads

Carboxylated magnetic beads (Beijing Bio Biology Company, China) were coated with rSj26GST according to the following method. In short, magnetic particles (particle size: 2 μm) were washed three times with 0.1 M pH6.0 MES buffer (2-Morpholineethanesulfonic acid hydrate, Sigma, USA), and then were re-suspended in MES buffer. EDC (1-ethyl-3-(dimethylaminopropyl)-carbodiimide hydrochloride, Sigma, USA) and NHS (N-hydroxysuccinimide, Sigma, USA) were added. The resulting mixture was incubated for 15 min at 25 °C while rotating. The beads were then washed three times with phosphate buffer saline (PBS, 0.01 M, pH 7.4). Subsequently, rSj26GST was incubated with the activated magnetic beads for 2 h at 37 °C while rotating. Thereafter, all the residual binding sites on the coated beads were blocked by bovine serum albumin (BSA) (1.5% final concentration, w/v). Finally, the beads were washed three times with Tris-buffer containing 0.05% Tween-20 (TBST pH7.4) and re-suspended in 0.1 M TBS (pH 7.4, with 0.1% BSA and 0.01% sodium azide). The magnetic beads coated with the antigens were stored at 4 °C until use.

2.6. Development of MEIA method

60 μL of magnetic beads (0.2 mg of magnetic beads coated with10 μg antigens) and 30 μL of diluted serum sample were added to per tube, wherein the serum sample was diluted in 0.1 M TBS (pH 7.4) (1:100 for human serum, 1:20 for mouse serum). The whole mixture was incubated with continuous shaking for 20 min at 37 °C, and then the desired product was collected as pellet by magnetic separation. Washing buffer (TBST) was added, and the washing treatment is repeated three times to remove unbound antibody. Subsequently, goat anti-mouse/human IgG conjugated to alkaline phosphatase (AP) (Proteintech, China) at a dilution of 1:2000, was added and incubates while rotating for 20 min at 37 °C. After washing, 100 μL of phenolphthalein monophosphate (PMP) was added and incubate while rotating for 20 min at 37 °C. Finally, the reaction was terminated by adding 300 μL of stopping reagent (0.05 mol/L Na_2CO_3), the absorbance at 550 nm was measured on a Serozyme I instrument (Merck Serono, Switzerland). Each serum sample was tested three times, and the arithmetic mean of the three results is used in the subsequent data analysis.

2.7. ELISA method

ELISA plates were coated at 4 °C overnight with 100 μL per well of 10 μg/ml rSj26GST and then washed. After adding 300 μL blocking buffer (TBST and BSA (1.5%), pH 7.4), the plates were incubated at 37 °C for 1.5 h and washed. Serum was diluted in 0.1 M TBS (pH 7.4) (1:100 for human serum, 1:20 for mouse serum), 100 μL was added to each well, and the plates were incubated at 25 °C for 1 h and then washed. AP conjugated goat anti-mouse/human IgG was added at a dilution of 1:2000 and incubated at 25 °C for 1 h before the plates were washed. The plates were developed with PMP substrate and the reaction stopped by adding 50 μL stopping reagent (0.05 mol/L Na_2CO_3) after 30 min. The plates were read at 550 nm, using an ELISA reader (Bio-Rad mod. 550). Each serum sample was tested in duplicate.

2.8. Data analysis

The P value is defined as $OD_{550\ nm}$ of positive control serum and N value is defined as $OD_{550\ nm}$ of negative control serum. The cut-off