

Clonorchis sinensis: glutathione *S*-transferase as a serodiagnostic antigen for detecting IgG and IgE antibodies

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Abstract

Human *Clonorchis sinensis* infection is endemic in East Asian countries. Glutathione *S*-transferases (GSTs) are anti-oxidant enzymes found in all living creatures as well as in trematodes. In this study, we examined the recombinant 26 kDa GST protein of *C. sinensis* (Cs26GST) for its serodiagnostic antigenicity toward IgG and IgE antibodies by ELISA and immuno-enhanced chemiluminescence, respectively. In IgG ELISA, recombinant Cs26GST showed 33.3% sensitivity and 100% specificity for trematode-infected human sera. In the case of the IgE antibody, recombinant Cs26GST showed 50.0% sensitivity and 93.2% specificity for clonorchiasis infection. We propose that the recombinant Cs26GST is a potent serodiagnostic antigen for detecting *C. sinensis*-specific IgG and IgE antibodies, and that it be best used as an antigenic cocktail in combination with other antigens.

Index Descriptors and Abbreviations: *Clonorchis sinensis*; GST, glutathione *S*-transferase (EC 2.5.1.18); Cs26GST, 26 kDa GST of *C. sinensis*; rCs26GST, recombinant Cs26GST; ECL, enhanced chemiluminescence; ELISA, enzyme-linked immunosorbent assay; IgG; IgE; serodiagnosis; antigen.

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1. Introduction

The adult *Clonorchis sinensis* flukes live in the human bile duct and provoke pathologic changes in hepatobiliary tissues, such as, epithelial hyperplasia and periductal fibrosis. Fluke infection has also been shown to promote the development of cholangiocarcinoma, from epidemiological and experimental data (Chung and Lee, 1976; Lee et al., 1994). Clonorchiasis, an infection associated with *C. sinensis* colonization, is endemic in East Asian countries and in overseas Chinese communities. In the communities affected, about seven million people are infected with *C. sinensis* (Crompton, 1999). Clonorchiasis is generally diagnosed by a microscopic examination of stools, however nowadays, it is not easy to collect stools for individual diagnosis and mass surveys.

The serodiagnosis of helminthic infections offers an alternative approach that overcomes this difficulty. A good antigenic protein is a prerequisite for serodiagnosis, and should offer high sensitivity and specificity, and be readily obtainable in high purity.

The glutathione *S*-transferase (GST) of *C. sinensis* (Cs26GST) is a cytosolic enzyme of molecular mass 26 kDa, that catalyzes the conjugation of reduced glutathione to electrophilic compounds (Hong et al., 2001). A GST isoenzyme, of molecular mass 28 kDa, is quite distinct from Cs26GST in terms of its molecular biological properties and antigenicity. This isoenzyme has been localized to the tegument and mesenchymal tissues of *C. sinensis* (Kang et al., 2001). GSTs have been suggested to play a role in the phase II defense line by detoxifying exogenous and endogenous bioreactive chemicals at the host-parasite interface (Hayes and Pulford, 1995). The GSTs of trematodes have been reported to be potent antigens in mammals. For example,

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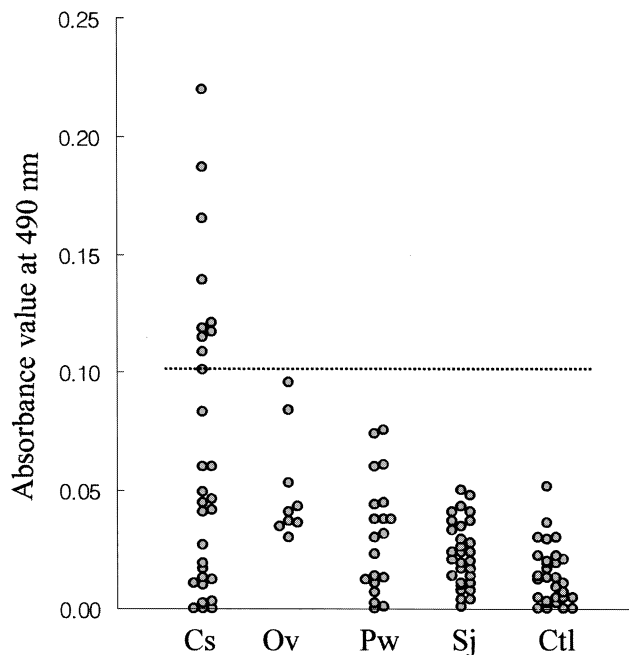


Fig. 1. Enzyme-linked immunosorbent assay of recombinant Cs26GST for IgG antibody in trematode-infected human sera. Cs, clonorchiasis sinensis; Ov, opisthorchiasis viverinii; Pw, paragonimiasis westermani; Sj, schistosomiasis japonicum; Ctl, uninfected control.

immunization with *Schistosoma mansoni* 28 kDa GST reduced the worm burden and female worm fecundity (Boulanger et al., 1991).

In the present study, we produced recombinant Cs26GST and evaluated its serodiagnostic usefulness for detecting IgG and IgE in cases of clonorchiasis.

The coding region of Cs26GST cDNA was amplified by PCR and subcloned into pET23b(+) (NOVAGEN, Germany). Bacterially overproduced recombinant Cs26GST (rCs26GST) was purified by GSH-agarose affinity column chromatography, as described previously (Hong et al., 2001).

A micro-ELISA plate (Costar, Cambridge, MA) was coated with 100 μ l of 1.5 μ g/ml rCs26GST. The human sera included in this study were parasitologically and serologically proven to be infected with respective parasites. The sera used were from 30 clonorchiasis sinensis patients, 9 opisthorchiasis viverinii patients, 20 paragonimiasis westermani patients, and 30 schistosomiasis japonicum patients. Thirty sera from helminth-free humans were also included as negative controls. Primary antibody was used at a dilution of 1:100 and secondary antibody, peroxidase-conjugated anti-human IgG at a dilution of 1:3000. Color was developed using *o*-phe-

nylene diamine (Sigma Chemical), and the absorbance was taken as the geometric mean of triplicate absorbances measured at a wavelength of 490 nm.

To detect the IgE antibody, rCs26GST was displayed in 12.5% SDS-PAGE and electro-transferred onto a PVDF membrane (Hybond-P, Amersham LIFE SCIENCE), and incubated in trematode-infected human sera at a 1:1–2 dilution overnight at room temperature. The blot was then washed twice with PBS/Tween 20 and incubated in peroxidase-conjugated goat anti-human IgE antibody (Cappel, St. Louis, MO) diluted 1:500 in PBS/Tween 20 for 1–2 h. After washing with 2 $\times$ SSC, the enhanced chemiluminescence (ECL) signal was generated and detected on Hyperfilm-ECL, as described by the manufacturer (Amersham Pharmacia Biotech UK, England).

Using rCs26GST to detect the IgG antibody in trematode-infected human sera by ELISA, a positive cut-off value at $A_{490} = 0.100$, gave a 33.3% positive rate in the sera of clonorchiasis patients. All sera tested from patients other than clonorchiasis patients proved negative at this cut-off value (Fig. 1). The specificity of rCs26GST for the IgG antibody was 100% by ELISA.

The immuno-ECL technique for rCs26GST was found efficient for detecting IgE antibody in trematode-infected human sera. rCs26GST-specific IgE was detected, in 50.0% of the sera from clonorchiasis patients, in 11.1% of the sera of opisthorchiasis patients and in 30.0% of the sera of paragonimiasis patients (Fig. 2). rCs26GST-specific IgE was not detected in the sera of schistosomiasis patients or in the sera of the uninfected humans used as controls. The specificity of rCs26GST for IgE was 93.2% by the immuno-ECL. No significant correlation was found between the levels of IgE and IgG antibodies specific for rCs26GST; only one of the 30 clonorchiasis patients had specific IgE and IgG antibodies.

Serum IgG antibody specific to *C. sinensis* in experimental rabbits increased 4–6 weeks after metacercarial infection and plateaued 12 weeks later (Lee et al., 1988). One of the factors influencing antibody production is the amount of antigen presented to the host immune system. In rabbits immunized with affinity purified *S. mansoni* GSTs, a lower level of Sm26GST than Sm28GST was suggested to have caused lower titers of IgG antibody against Sm26GST than against Sm28GST (Henkle et al., 1990). Cs26GST is a relatively minor cytosolic enzyme and is expressed at approximately one-fourteenth of the level of Cs28GST (Kang et al., 2001). In mice rCs26GST was found to be immunogenic and to elicit strong IgG antibody production (Hong et al., 2001), which sug-

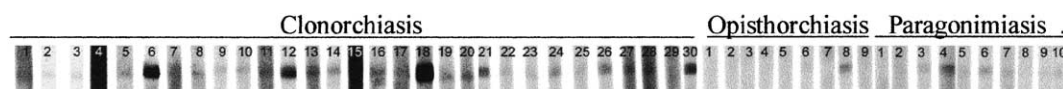


Fig. 2. IgE antibody reacting to recombinant *C. sinensis* 26 kDa GST in trematode-infected patients' sera. The blot was incubated in peroxidase-conjugated anti-human IgE antibody. The signal was detected by using enhanced chemiluminescence reagents.

gested that the smaller amount of Cs26GST in *C. sinensis* perhaps explains the late production of specific IgG antibody in clonorchiasis patients.

In the serodiagnosis of human clonorchiasis, ELISA showed a sensitivity of over 75% and a somewhat lower specificity when used upon a crude worm extract as antigen (Yong et al., 1991). Efforts to improve the quality of diagnostic antigens, have characterized antigenic proteins from *C. sinensis*. Antigenic proteins such as Cs31 (Yong et al., 1998) and CsK2 (Kim, 1998) appeared to offer satisfactory specificity but inadequate sensitivity of ELISA for clonorchiasis. rCs26GST used as an antigenic protein, also showed a low sensitivity for specific IgG antibodies in the serodiagnosis of human clonorchiasis.

In cases of animal and human schistosomiasis, IgE is a humoral factor of an antibody-dependent, cell-mediated cytotoxicity system, which targets the parasite larvae. Immunization with Sm28GST induces IgE antibody production, and this results in a reduced worm burden and parasite fecundity (Boulanger et al., 1991; Capron and Capron, 1994). In experimental rats, serum IgE antibody, specific to *C. sinensis*, increased abruptly only 2 weeks after metacercarial infection (Min et al., 1980), and in clonorchiasis patients, specific IgE antibody was found to be significantly elevated (Min and Soh, 1983). Of the antigenic proteins from *C. sinensis*, proteins with a molecular mass of 43, 34, 28, and 26 kDa were found to be reactive to IgE antibodies in clonorchiasis patient sera (Hong et al., 1997). Moreover, in the present study, the reactivity of rCs26GST to IgE antibody in the patient's sera, as detected by immuno-ECL clonorchiasis, was sensitive enough to be used for the serodiagnosis of human clonorchiasis. The GSTs of the house dust mite and of the German cockroach, recognizing IgE antibodies in the sera of allergic patients, were proposed to act as potent serodiagnostic reagents, though they showed relatively low sensitivities (O'Neill et al., 1994; Arruda et al., 1997).

It seems that defined proteins of *C. sinensis*, when used as a single antigen, offer limited serodiagnostic potential for human clonorchiasis. In this context, rCs26GST could be used as an antigenic molecule for the serodiagnosis of human clonorchiasis. We suggest that rCs26GT could be used as a component of an antigen cocktail of recombinant proteins for the serodiagnosis of human clonorchiasis (Kim et al., 2001).

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