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Specificity of isoelectric focusing-purified antigens in the diagnosis of human cysticercosis

Abstract Specific antigens were isolated from the cystic fluid of larval Taenia solium by preparative isoelectric focusing (PIEF). A total of 20 fractions were produced by a rotating ampholine column with pI 3–10 ampholytes. The specificity of each fraction (F) was tested by double-antibody enzyme-linked immunosorbent assay (ELISA) using antisera from patients suffering from cysticercosis or one of six other parasitic diseases. F8–F15 cross-reacted strongly with sera from patients with hydatidosis. F9 and F10 also cross-reacted with the antisera against ascariasis and F15, with antisera against angiostrongylosis. However, F16 and F17 were highly specific as they yielded no cross-reaction with any of the heterologous antisera. PIEF is a good method for the production of specific antigens from larval T. solium because it is easy to perform and relatively inexpensive to run.

Introduction

Immunodiagnosis of human cysticercosis is confronted with numerous technical problems. A major difficulty is the unavailability of a specific antigen that will not cross-react with closely related heterologous antibodies (Flisser et al. 1982; Cheng and Ko 1991). For example, crude somatic antigens of larval Taenia solium invariably cross-react with anti-hydatidosis serum. Pammeter et al. (1992) have suggested that even schistosomiasis may interfere with the immunodiagnosis of neurocysticercosis. The underlying cause is the presence of too many conserved antigens in both the membranous wall and the cystic fluid of larval cestodes. Our recent study has also shown that unlike those of the nematodes, the excretory/secretory products of larval T. solium lack specific immunodominant antigens (Ko and Ng 1998).

Tsang et al. (1989) first reported the recognition of seven specific glycoprotein bands by sera of patients in immunoblots of whole-cyst extracts. Plancarte et al. (1994) attempted to purify one of the bands by elution from the gel. However, the glycoprotein antigens have not been mass-produced or purified. McManus et al. (1991) produced beta-galactosidase fusion peptides of Mr ranging from 135–140 kDa that were recognized by sera of patients. However, the specificity and sensitivity of the expressed products have yet to be determined.

Therefore, in view of the above observations, it is necessary to find a relatively easy method for the isolation of specific antigens from T. solium. Ko and Ng (1998) reported that F15, a fraction isolated by isoelectric focusing, showed a high degree of specificity in diagnosing porcine cysticercosis. In this study the specificity of the isolated fractions in diagnosing human cysticercosis was evaluated by enzyme-linked immunosorbent assay (ELISA).

Materials and methods

Antigen and antiserum preparation

Intact cysts (Cysticercus cellulosae) were removed from the muscles of naturally infected pigs within 24 h of slaughter in the Kennedy Town abattoir, Hong Kong. Crude whole-cyst antigens were prepared by homogenization of 1 g of cysts in phosphate buffer following the method of Cheng and Ko (1991). Cystic fluid was obtained by aspiration with a 27-gauge hypodermic needle. Antigen samples were desalted by passage through a P6DG column (Biorad) and then concentrated by a sample concentrator (Speed-Vac). The protein concentrations of samples were determined by a protein assay kit (Biorad). After preparation the antigens were frozen at −20 °C before use.

Blood samples were collected by doctors from patients admitted into various hospitals in Hong Kong. The serum was separated by centrifugation and then frozen before it was forwarded to the Parasitology Laboratory, Department of Zoology, University of Hong Kong. The diagnoses of the parasitic diseases were confirmed according to two or more of the following parameters: clinical
manifestations, computerized tomography scanning, serological testing, muscle biopsy, and fecal examination. Heterologous infections included angiostrongyliasis, ascariasis, trichuriasis, clonorchiasis, and hydatidosis.

Due to the limited quantity of each isolated fraction, it was not possible to test a very large sample of sera from various patients. Therefore, from a pool of infected sera stored in the parasitology laboratory (at least ten per disease), only the sample with the highest titer was selected for the specificity study. Negative control sera were obtained from members of our laboratory staff.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectric focusing

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970) in a Mini-Protean II cell (Biorad). The gels were stained using a silver staining kit (Sigma). Molecular mass was determined by Dalton Mark VIII markers (Sigma).

Preparative isoelectric focusing (PIEF) was performed in a rotating ampholine column (Rotofor, Biorad). It was connected to a refrigerated recirculator (model 4860, Biorad) and a microprocessor-controlled power supply (3000xi, Biorad). In brief, 100 mg of cystic fluid antigens were separated into 20 fractions with 1–2% pIs. Ampholytes in the samples were removed by batch deionization using Bio-Rex AG901-X8 resin (Biorad).

Analytical isoelectric focusing (AIEF) was performed in a Multiphor Cell (LKB) with precast polyacrylamide gels (pI 3–10, 4–6.5). Electrofocusing was carried out at 2000 and 35 W. The gels were stained using a silver staining kit (Sigma).

Double-antibody ELISA

The double-antibody-IgG ELISA was performed according to Ko and Yeung (1991). The optimal concentrations of antigen, antibodies, and enzyme conjugated were first determined by checkerboard titration. Flat-bottom polystyrene microtiter plates (Linbro, Flow Lab) were coated overnight at 4 °C with purified F antigens (5 μg ml⁻¹ in 100 μl/well) diluted in coating buffer (0.1 M carbonate-bicarbonate buffer, pH 9.6). Coated plates were washed five times by an automatic plate washer (Dynatech) using 0.9% NaCl and 0.05% Tween 20. The test serum (diluted 1:100, v/v) was added. The plate was kept overnight at 4 °C followed by washing, goat anti-human IgG horseradish peroxidase (HRPO; heavy- and light-chain-specific, Cappel, diluted 1:8000, v/v) was added. After incubation for 2 h at 37 °C followed by washing, orthophenylenediamine (OPD) was added to the wells. The reaction was stopped after 30 min of incubation, 0.05 ml of concentrated H₂SO₄ was added. The plate was kept overnight at 4 °C and then washed before the addition of the substrate, orthophenylenediamine (OPD). After 30 min of incubation, 0.05 ml of concentrated H₂SO₄ was used to stop the reaction. The plates were read by an automatic ELISA reader (MR 710, Dynatech) at 490 (test filter) and 410 nm (reference filter). Absorbance values (OD) that were greater than three times the mean OD recorded for the positive control were considered as positive.

Results

The SDS-PAGE study showed that the protein profile of the various fractions (F) separated by PIEF is basically

Fig. 2A, B  Results of testing of sera samples from patients by double-antibody IgG ELISA using A F8 and B F9 as coating antigens. Absorbance is expressed in optical density (OD). The OD ratio is obtained by division of the positive control OD by the negative control OD. The positive cut-off value is 3 times the mean OD recorded for the positive control. Solid bars represent OD readings; shaded bars represent OD ratios. Sera used were: 2, 19, 28 – cysticeriosis; Ag – angiostrongyliasis; Tr – trichinellosis; Tr – trichuriasis; As – ascariasis; Cl – clonorchiasis; Hy – hydatidosis

Fig. 3A–H  Results of double-antibody IgG ELISA using A F10, B F11, C F12, D F13, E F14, F F15, G F16, and H F17 as coating antigens. Absorbance is expressed in optical density (OD). The OD ratio is obtained by division of the positive control OD by the negative control OD. The positive cut-off value is 3 times the mean OD recorded for the positive control. Solid bars represent OD readings; shaded bars represent OD ratios. Sera used were: 2, 19, 28 – cysticeriosis; Ag – angiostrongyliasis; Tr – trichinellosis; Tr – trichuriasis; As – ascariasis; Cl – clonorchiasis; Hy – hydatidosis.

Fig. 1 SDS-PAGE profile of F8–F17 isolated from cystic fluid of larval Taenia solium by the isoelectric focusing method. Lane m is the molecular marker (from top to bottom): 97.4, 68, 43, 29, 18.4, and 14.4 kDa