Use of *Taenia crassiceps* Cysticercus Antigen Preparations for Detection of Antibodies in Cerebrospinal Fluid Samples from Patients with Neurocysticercosis (*Taenia solium*)

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Neurocysticercosis (NC), caused by the larval form of *Taenia solium*, is considered to be an endemic disease in underprivileged regions such as Latin America, Asia, Africa, China, and Indonesia (2, 16, 23, 26), and it represents an important public health problem in these regions.

The diagnosis of NC is based on clinical and laboratory criteria and on imaging examinations such as axial computed tomography and magnetic resonance imaging, which are efficient methods for the visualization of cysticerci and of an inflammatory response but are very expensive and inaccessible to most of the affected population (4).

Although the immunological tests used for the diagnosis of cysticercosis have been considerably refined, the selection of an adequate antigen is required to improve diagnostic efficiency, and this continues to be an area of interest.

Earlier studies have demonstrated the importance of the use of purified extracts obtained mainly from the glycoprotein fractions of *T. solium* for the detection of antibodies against cysticercus antigens in immunological assays (6, 7, 10, 11, 22). The difficulty of obtaining parasites from naturally infected pigs for the isolation of *T. solium* antigen continues to be a problem. Therefore, our group has been studying the use of antigens obtained from cysticerci of the *Taenia crassiceps* ORF strain (1, 25). The objective of the present study was to determine the efficiency of antigen fractions purified from *T. crassiceps* cysticerci in the detection of specific antibodies in cerebrospinal fluid (CSF) samples from patients with NC.

**Parasites and antigens.** Vesicular fluid antigen was obtained from the larval form of *Taenia crassiceps* (VF-Tcra) ORF strain (8) as described previously (25). The purified fractions of concanavalin A (ConA-Tcra) were obtained by affinity chromatography with lectin. Total antigen was subjected to gel filtration on a PD-10 Sephadex G-25M column, purified by affinity chromatography on a ConA-Sepharose 4B column (Pharmacia Fine Chemicals) equilibrated with Tris-HCl buffer (pH 7.4) (0.5 M NaCl, 0.05 M Tris), and eluted with 0.2 M α-methylm-annonypyransoxide solution. The protein peak was detected by spectrophotometry at 280 nm and concentrated by ultrafiltration through a YM-10 membrane (Amicon).

For isolation of the gp-Tcra fraction, the VF-Tcra antigen containing 14 mg of protein per ml was treated with sample buffer (0.014 M 2-mercaptoethanol, 2% sodium dodecyl sulfate (SDS)), heated for 5 min, and applied to the gel (Prep-Cell model 491; Bio-Rad Laboratories Inc., Richmond, Calif.). The material was eluted after preparative SDS-polyacrylamide gel electrophoresis (PAGE) and the protein peaks were detected by spectrophotometry at 280 nm. The fractions were individually processed by SDS-PAGE (12% polyacrylamide) and silver staining to select fractions with molecular masses of 18 and 14 kDa. The fractions were pooled and dialyzed against 0.01 M ammonium bicarbonate buffer for 48 h and against phosphate-buffered saline (PBS) (pH 7.2) for an additional 24 h. The antigen was concentrated with polyethylene glycol 8000 (Sigma Chemical Co., St. Louis, Mo.), yielding the *T. crassiceps* glycoprotein antigen extract, gp-Tcra.

**Samples.** The patients were selected on the basis of the General NC Investigation Protocol of the Hospital of the Faculty of Medicine, University of São Paulo, and the protocol was approved by the Ethics Committee for the Analysis of Research Projects of the Clinical Director’s Office of the Hospital (approval no. 072/97), according to Resolution 196/96 of the National Health Council, Ministry of Health, Brasília, Brazil.

(i) NC group. We analyzed 29 CSF samples, from patients with a clinical and laboratory diagnosis of NC, obtained from...
the CSF Laboratory. Also included in this study were 52 CSF samples classified by imaging examinations (15), with 7 (13.46%) samples being classified as type II (intact cysts), 12 (23%) being classified as type III (cysts associated with an inflammatory process and/or degenerating cysts), 13 (25%) being classified as type IV (nodular calcifications), 17 (32.69%) being classified as having cysts in more than one phase of evolution (mixed type), 1 (1.92%), being classified as having cysts presenting ventricular localization, and 2 (3.84%) from patients for whom no imaging results were obtained.

(ii) Control group. Twenty-nine CSF samples from patients with a negative clinical and laboratory diagnosis of NC were obtained from the CSF Laboratory.

Enzyme-linked immunosorbent assay (ELISA) procedure.
(i) T. crassiceps antigens. Ninety-six-well plates (Nunc) were sensitized with VF-Tcra (2 µg/ml), ConA-Tcra (0.43 µg/ml), or gp-Tcra (20 µg/ml) with 0.02 M carbonate-bicarbonate buffer (pH 9.6) for 18 h in a humidified chamber at 4°C. The plates were blocked with 5% skim milk (Molico skim milk; Nestlé, Araçatuba, São Paulo, Brazil) in 0.01 M PBS (pH 7.2) containing 0.05% Tween 20 (Merck KGaA, Darmstadt, Germany) (PBS-T). CSF was diluted 1:2, and peroxidase-labeled sheep anti-human immunoglobulin G (IgG; Biolab Diagnostics SA, Jaguarépagua, RJ, Brazil) was used as the conjugate. The enzymatic reaction was developed with the chromogenic substrate tetramethylbenzidine-hydrogen peroxide (Bio-Rad Laboratories Inc.) for 15 min in the dark and blocked with 4 N sulfuric acid. The labeling intensity was quantified with a plate reader at 450 nm (Diagnostics Pasteur, Strasburg-Schiltigheim, France).

All incubations were carried out for 30 min at 37°C. Between the steps of sample, conjugate, and substrate incubation, the plates were washed in an automatic washer with four cycles of saline solution containing 0.05% Tween 20. All plates contained a positive, negative, and blank (no-sample) control.

The cutoff was determined based on the analysis of the results of the control group. Samples with an optical density equal to or higher than the cutoff were considered to be positive. The reactivity index was calculated by dividing the optical density of the samples by the cutoff obtained for each antigen.

(ii) T. solium antigen. The commercial BioELISA Neurocistis kit (Biolab Diagnóstica SA, Jaguarépaguá, RJ, Brazil) with T. solium antigen was used for the detection of anti-T. solium antibodies as specified by the manufacturer.

Immunoblotting procedure. VF-Tcra, ConA-Tcra, and gp-Tcra were subjected to SDS-PAGE (13), and then transferred to a 0.22-µm-pore-size nitrocellulose membrane (Millipore Corp., Bedford, Mass.) (VF-Tcra) or 0.1-µm-pore-size polyvinylidene fluoride membrane (Millipore Corp.) (ConA-Tcra and gp-Tcra) in an electrophoretic cuvette (TE 42 Transphor Unit; Amersham Pharmacia Biotech) for 16 to 18 h at 4°C.

The membranes were cut into 3-mm strips, washed three times with PBS-T for 10 min, and blocked with 5% skim milk in PBS-T (diluting solution) for 2 h. After being washed, the CSF samples were diluted 1:4 and incubated overnight at 4°C under constant shaking. For VF-Tcra antigen, the blots were incubated with biotin-labeled goat anti-human IgG as conjugate and peroxidase-labeled avidin (Cambridge Biotech Co., Cambridge, Mass.) for 2 h and the enzymatic reaction was developed with 0.017% dianaminobenzidine and 5% H₂O₂. For the ConA-Tcra and gp-Tcra antigens, alkaline phosphatase-labeled human anti-IgG conjugate (Bio-Rad Laboratories Inc.) was added and the enzymatic reaction was developed with 5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium (BCIP/NBT) (Sigma). Positive, negative, and background (no-sample) controls were included in each test.

ELISA results. (i) T. solium. Four samples from the NC group showed undetermined results, and two other samples were negative for anti-T. solium antibodies.

(ii) T. crassiceps. ELISA results obtained for the CSF samples from the NC and control groups using T. crassiceps antigen are shown in Fig. 1. The 29 samples from the NC group showed reactivity against the ConA-Tcra antigen. The 28 samples assayed with the VF-Tcra antigen and the 15 samples assayed with the gp-Tcra antigen were also reactive.

ELISA results obtained with CSF samples from patients classified by imaging examination by the method of Machado et al. (15) using the ConA-Tcra antigen are shown in Fig. 2. Of the 52 CSF samples, 2 were negative when assayed with T. solium total saline antigen and VF-Tcra (data not shown) and were also negative for the ConA-Tcra antigen (type III and type IV). Another sample also presented a negative result (type III). All three samples were negative when assayed with the gp-Tcra antigen.

The NC and control groups showed 100% sensitivity and specificity in the ELISAs.

Immunoblotting results. Figure 3 shows the immunoblots of the CSF samples from the NC group assayed with T. crassiceps antigen. The 18- and 14-kDa peptides were identified only in NC-positive CSF samples (frequency of 100%).

Comparison of methods. The replacement of T. solium antigen with T. crassiceps antigen was initially evaluated by Larralde et al. (14), who showed that, in addition to its significant reproducibility, T. crassiceps antigen was efficient in the detection of antibodies in CSF samples. The application of VF-Tcra was also evaluated by dot ELISA and showed 100% specificity in CSF samples from patients with NC (24). Vaz et al. (25), using VF-Tcra in immunoblots of CSF samples from patients with NC, obtained results indicating that the parasite possesses epitopes common to T. solium cysticerci in an amount sufficient to be used in immunological tests. Pinto et al. (18), using serum from swine cysticercosis, obtained 76.9% sensitivity and 97% specificity in ELISA using T. crassiceps antigen.

T. solium antigen demonstrated a lower sensitivity (79.3%) in ELISA of CSF samples from the NC group than in ELISA of heterologous extracts (100%). This result can be explained by the fact that different antigen preparations were used, since T. solium total extract has a smaller number of low-molecular-mass peptides, mainly lower than 20 kDa, the size of the main components of T. crassiceps extracts.

Some authors using T. solium glycoprotein extracts obtained sensitivity and specificity similar to those reported here for T. crassiceps antigen (9, 12, 19, 20, 22).

The VF-Tcra and ConA-Tcra antigen extracts showed a complex antigen composition in immunoblots, impairing the interpretation of the results. When the gp-Tcra antigen was used, however, only the 18- and 14-kDa peptides, which are specific for the diagnosis of NC (1, 5), were identified, thus facilitating the interpretation of the results. The reactivity...
against low-molecular-mass peptides (≤20 kDa) found in T. crassiceps antigen extracts by immunoblotting was also observed in T. solium antigen extracts (22).

Some authors have suggested that the detection of antibodies against low-molecular-mass peptides may be associated with the evolutive phase of the parasite. Michault et al. (17), using a T. solium extract, identified antibodies recognizing the 14-kDa peptide in patients in the active phase of the disease.

Simac et al. (21) characterized the 13- and 14-kDa fractions present in T. solium total extract as potential markers for the diagnosis of NC in patients with vesicles revealed by computed tomography. Analysis of a 10-kDa protein (subunit of the 150-kDa protein) in T. solium vesicular fluid by immunoblotting and immunoprecipitation revealed 84.6% reactivity (27). Chung et al. (3), using a 10-kDa T. solium recombinant protein, obtained 98% specificity; they suggested the use of this peptide to

FIG. 1. ELISA results expressed as reactivity index for the detection of anti-T. solium antibodies in CSF samples from the NC and control groups assayed with T. solium (Tso), vesicular fluid (VF-Tcra), Con A (ConA-Tcra), and T. crassiceps glycoprotein (gp-Tcra) antigen extracts.

FIG. 2. ELISA results expressed as reactivity index for the detection of anti-T. solium antibodies in 52 CSF samples from different evolutive phases of the disease classified as suggested by Machado et al. (15) and assayed with ConA-Tcra antigen extract. The column on the right shows results from patients for whom no imaging results were obtained.
identify patients in the active phase of the disease. In the present study, antibodies at levels close to the cutoff were detected only in two patients classified as type III and in one classified as type IV, while in the remaining patients antibodies were identified with the ConA-Tcra glycoprotein extract only, i.e., an antigen not associated with the developmental stage of the parasite. It should be noted that the patients studied here had been previously monitored for 2 to 10 years and those classified as being in the phase of calcification were in the early phase of this process.

T. crassiceps cysticercus antigen extracts represent an important alternative source for antigens used in immunological tests. The isolation of antigen fractions with a molecular mass below 20 kDa may improve the specificity of the tests, especially in serum samples for which cross-reactions and nonspecific reactions have been frequently reported (11, 22).

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REFERENCES