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Cysticercosis Immunodiagnosis Using 18- and 14-Kilodalton Proteins from *Taenia crassiceps* Cysticercus Antigens Obtained by Immunoaffinity Chromatography

Noeli Maria Espíndola,[1] Alberto Hiroshi Iha,[1] Irene Fernandes,[2] Osvaldo Massaiti Takayanagui,[3] Luís dos Ramos Machado,[4] José Antônio Livramento,[4] Antônio Augusto Mendes Maia,[5] José Mauro Peralta,[6] and Adelaide José Vaz[1]*

Laboratório de Imunologia, Faculdade de Ciências Farmacêuticas, Universidade de São Pulo, São Paulo,[1] Laboratório de Imunopatologia do Instituto Butantan, São Paulo,[2] Departamento de Neurologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto,[3] Faculdade de Medicina, Universidade de São Paulo, São Paulo,[4] Departamento de Ciências Básicas, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga,[5] and Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro,[6] Brazil

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Monoclonal antibodies (MAb) against *Taenia crassiceps* and *Taenia solium* cysticerci were produced and showed cross-reactivity with a 14-kDa protein from *T. solium* and with 18- and 14-kDa proteins from *T. crassiceps*. These MAbs and antibodies from cerebrospinal fluid (CSF) as well as serum samples from patients with neurocysticercosis (NC) reacted with 18- and 14-kDa *T. crassiceps* proteins purified by immunoaffinity chromatography using a Sepharose column coupled with MAbs (anti-excretory/secretory or anti-vesicular fluid antigens). Immunoaffinity-purified 18- and 14-kDa proteins were used in the design of a diagnostic enzyme-linked immunosorbent assay (ELISA) to detect antibodies in 23 CSF and 20 serum samples from patients with NC, showing 100% sensitivity. The test specificity was determined using 42 noninflammatory CSF samples and 70 inflammatory CSF samples from patients with other neurological disorders (OND), showing 100% and 99.1% (confidence interval, 97.3% to 100%) specificity, respectively. A false-positive CSF sample result in the OND group was from a human immunodeficiency virus-positive patient with meningoencephalitis. By using serum samples from 194 healthy individuals, the specificity was 100%. Analysis of an additional 16 serum samples from individuals with other parasitic diseases (13 with intestinal parasitosis and 3 with schistosomiasis) showed negative results. Three (10%) serum samples from patients with hydatidosis were positive in our ELISA and in ELISA with *T. solium* cysticerci antigens. Two of them were also positive by immunoblotting. The use of 18- and 14-kDa *T. crassiceps* immunoaffinity-purified proteins for detection of anti-cysticercus antibodies in CSF and/or serum samples using an ELISA system showed a good performance and high specificity for serum samples, dispensing with the use of confirmatory tests, such as immunoblotting, for checking specificity.

Neurocysticercosis (NC) is caused by *Taenia solium* cysticerci in the central nervous system. Serological tests are helpful for the precise diagnosis because they confirm or supplement clinical and laboratorial diagnosis based on brain image investigation (12).

Although several serological methods have been evaluated to date, these tests still present problems. False-negative results can be obtained in cerebrospinal fluid (CSF) and serum samples from proven NC patients, and false-positive results have been reported for patients with other pathologies, particularly other parasitic diseases (11), and even for healthy individuals (1, 2). The detection of serum antibodies is impaired by cross-reactivity with other parasites, mainly when crude antigens are used. These data point out a need for the use of purified preparations to circumvent these problems.

Glycoprotein fractions obtained from *T. solium* cysticerci antigen by lentil-lectin (*Lens culinaris*) affinity chromatography were considered efficient for the serological diagnosis of NC (8, 17, 23). However, its use has been limited due to the assay technology, high cost, and complexity and length of the antigen purification process. Fractions with molecular masses below 15 kDa purified by affinity chromatography with anti-*T. solium* monoclonal antibody (MAb) specifically detected anti-*T. solium* antibodies in samples from NC patients (4, 12). The limited source of *T. solium* cysticerci hampers the large-scale production of specific antigens by these purification methods (24).

More recently, the use of recombinant proteins or even synthetic peptides from *T. solium* has been reported, and investigations are under way (5, 9, 10). Probably due to the complexity of the immune response in NC patients, a mixture of several specific and well-characterized proteins will give the desired levels of sensitivity and specificity.

On the other hand, the method for obtaining antigenic extracts from *Taenia crassiceps* cysticerci and their cross-reactivity with *T. solium* cysticerci antigens (13, 15, 27, 28) made them an interesting alternative antigen source for diagnosis (2, 21, 22) and immunological investigation of cysticercosis (3, 7, 18).

Vesicular fluid of *T. crassiceps* has been efficiently used in

* Corresponding author. Mailing address: Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Av. Lineu Prestes, 580, Bloco 17, Laboratório de Imunologia Clínica, CEP 05508-900, São Paulo, SP, Brazil. Phone: 55 11 30913662. Fax: 55 11 38132197. E-mail: ajvaz@usp.br.

TABLE 1. Human samples of NC

Sample type	No. of samples positive by criteria:		Total no. of samples
	Neuroimaging, clinical picture, and positive immunological tests	Clinical picture and positive immunological tests	
Serum	20[a]	0	20
CSF	23[b]	9	32

[a] Two samples had negative paired CSF serology.
[b] Five samples had negative paired serum serology.

the diagnosis of cysticercosis, and the 18- and 14-kDa fractions from *T. crassiceps* have been considered specific for the immunodiagnosis of NC using an immunoblotting assay (1). High-molecular-weight peptides have been associated with cross-reactivity when human (1) and swine (21) serum samples were assayed.

Purified proteins from *T. crassiceps* antigens and their use in a simple test, such as the enzyme-linked immunosorbent assay (ELISA) format, may contribute to the improvement of the specificity of immunological tests applied for clinical diagnostic and surveillance studies of human and pig cysticercosis infection.

In this study, we report a simple method for the purification of native specific proteins of *T. crassiceps* cysticerci antigens, using two anti-*T. crassiceps* MAbs selected from a panel of MAbs cross-reacting with *T. solium* and *T. crassiceps* antigens in an ELISA to detect antibodies in CSF and serum samples from NC patients.

MATERIALS AND METHODS

Samples. Serum and CSF samples were obtained from patients attending the Faculty of Medicine Hospital at the University of São Paulo, *campi* São Paulo and Ribeirão Preto, Brazil. Twenty-three CSF and 20 serum samples from patients with NC were used. These patients had NC diagnosis confirmed by imaging exam (computed tomography and/or magnetic resonance imaging) and clinical and immunological data. Additionally, 9 CSF samples from patients with clinical findings and positive immunological tests for NC were also tested (Table 1).

The CSF control group (CG) consisted of 112 CSF samples obtained from patients with other neurological disorders (OND). Forty-two of these CSF samples did not show laboratory alteration and had the diagnosis of meningitis excluded (noninflammatory CSF), while 70 were inflammatory CSF, including viral or bacterial meningitis (19 samples), neurosyphilis (6 samples), cytomegalovirus meningoencephalitis (4 samples), neurotoxoplasmosis (1 sample), human T-cell leukemia virus type 1-associated myelopathy/tropical spastic paraparesis (1 sample), and human immunodeficiency virus (HIV)-associated meningoencephalitis (39 samples) (Table 2).

The CG consisted of 194 serum samples from apparently healthy individuals and 46 samples from patients with other parasitic diseases, including 13 intestinal parasites (*Giardia lamblia* [2 samples], *Entamoeba histolytica* [3 samples], *Ascaris lumbricoides* [2 samples], and *Strongyloides stercoralis* [6 samples]) and 33 systemic parasites (hydatidosis, *Echinococcus granulosus* [30 samples], and schistosomiasis, *Schistosoma mansoni* [3 samples]) (Table 2).

This study was approved by the Ethics Committee for the Analysis of Research Projects of the FCF/USP (approval 188/2003) and complied with Resolution 196/96 of the National Health Council of the Brazilian Ministry of Health.

Experimental animals. Animal manipulations were approved by the Ethics Committee for Experimental Animals of the FCF/USP (project 13/2003), adopted by the Brazilian Committee for Experimental Animals.

Parasites and antigens. *T. crassiceps* and *T. solium* cysts were obtained as described by Espíndola et al. (7). Four different antigen preparations were used: two from *T. crassiceps* cysticerci and two from *T. solium* cysticerci. Phenylmethylsulfonyl fluoride (Sigma Chemical Co., St. Louis, Mo.) was added to each preparation, at a final concentration of 0.4 mM, to inhibit protease activity. Excretory/secretory *T. crassiceps* (ES-*T. crassiceps*), vesicular fluid of *T. crassiceps* (VF-*T. crassiceps*), or total *T. solium* (T-*T. solium*) antigens were produced according to the method of Espíndola et al. (7). Vesicular fluid of *T. solium* (VF-*T. solium*) was prepared from intact cysticerci of *T. solium*. After at least 10 washes with phosphate-buffered saline (PBS, 0.01 M; 0.0075 M Na_2HPO_4, 0.025 M NaH_2PO_4, 0.15 M NaCl, pH 7.2), vesicular fluid was aspirated from the cysts with a syringe coupled with a 13- by 0.4-mm needle. The fluid was centrifuged at 15,000 × *g* for 60 min at 4°C. The supernatant was sonicated (at 20 kHz and 1 mA, for four 30-s periods in an ice bath) and then centrifuged at 15,000 × *g* for 60 min at 4°C. The supernatant was collected corresponding to the VF-*T. solium* antigen.

Monoclonal antibody production. BALB/c mice were immunized with each antigenic preparation (ES-*T. crassiceps*, VF-*T. crassiceps*, VF-*T. solium*, and T-*T. solium*), and MAbs were obtained as described by Espíndola et al. (7). The MAb isotypes were determined using a commercial kit (mouse monoclonal antibody isotyping reagents; Sigma).

Antigen purification by immunoaffinity chromatography. MAbs were coupled to CNBr-Sepharose 4B according to the manufacturer's instructions (Amershan Pharmacia Biotech, Piscataway, N.J.), using 10 mg of anti-VF-*T. crassiceps* antigen MAb or anti-ES-*T. crassiceps* antigen MAb per 3 g of CNBr-Sepharose 4B. A total of 14 mg of protein from VF-*T. crassiceps* antigen was applied to the column. Elution of unbound protein was monitored by UV monitor (Bio-Rad Laboratories, Inc., Hercules, CA). After column washes with borate/saline buffer (0.1 M H_3BO_3, 0.1 M $Na_2B_4O_7$, and 1.0 M NaCl, pH 8.5, diluted 1:20 in 0.15 M NaCl), desorption buffer (0.2 M glycine and 0.15 M NaCl, pH 2.8) was applied to elute the bound protein. Fractions were immediately neutralized with 1.0 M Tris (Tris hydroxymethylaminoethane) solution, concentrated, dialyzed against borate/saline buffer (pH 8.5), using a YM-10 membrane (Millipore Corp.), and then stored at −20°C.

SDS-PAGE, IB, and ELISA. The nonpurified or MAb immunoaffinity chromatography-purified VF-*T. crassiceps* antigens were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by following the method described by Laemmli (14). The antigens were solubilized with sample buffer (0.01 M Tris-HCl, pH 6.8, containing 2% SDS, 5% 2-mercaptoethanol, and 10% glycerol) at 100°C for 5 min and separated electrophoretically on a 15% polyacrylamide gel. For immunoblotting (IB) analysis, the separated antigens were electrophoretically transferred to a 0.22-μm-pore-size membrane of polyvinylidene difluoride (Millipore Corp., Bedford, Mass.). The membranes were blocked by treatment with 5% skim milk (Molico skim milk; Nestlé, Araçatuba, SP, Brazil) in PBS for 2 h, washed in PBS containing 0.05% Tween 20 (Merck, Schudart, Munich, Germany), and then incubated for 18 h at 4°C with MAbs diluted 1:1,000 or sera or CSF diluted 1:50 and 1:10, respectively, in 1% skim milk in PBS. After further washes, strips were incubated for 1 h with goat anti-human immunoglobulin G (IgG)-biotin/avidin peroxidase (Sigma) or goat anti-mouse IgG-alkaline phosphatase (Bio-Rad Laboratories, Inc.) conjugates. After additional washes, the antigen-antibody complexes were developed by

TABLE 2. Human CG samples

Sample type	No. of samples from:					Total no. of samples
	Healthy individuals	Patients with OND and CSF type:		Patients with other parasites		
		Noninflammatory	Inflammatory	Intestinal[a]	Systemic[b]	
Serum	194	0	0	13	33	240
CSF	0	42	70	0	0	112

[a] Intestinal parasites were as follows: *G. lamblia* (2 samples), *E. histolytica* (3 samples), *A. lumbricoides* (2 samples), and *S. stercoralis* (6 samples).
[b] Thirty samples were from patients with hydatidosis, and three samples were from patients with schistosomiasis.

TABLE 3. Specificity by immunoblotting with VF-*T. crassiceps* and T-*T. solium* antigens using anti-*T. crassiceps* and anti-*T. solium* MAbs

MAb	Clone(s)	Protein(s) detected by immmunoblotting with antigen:	
		VF-*T. crassiceps*	T-*T. solium*
Anti-ES-*T. crassiceps*	ES[a]	18 and 14 kDa	14kDa
Anti-VF-*T. crassiceps*	VF	18 and 14 kDa	14kDa
Anti-VF-*T. solium*	A3, G12	18 and 14 kDa	14kDa
Anti-T-*T. solium*	A6, B4, B11, E10	18 and 14 kDa	14kDa

[a] IgG2b isotype (all of the others were IgG1).

incubation with an appropriate substrate: 4-chloro-1-naphthol (Sigma) predissolved in methanol (20% of the volume) and then diluted to 0.05% with Tris-buffered saline (0.01 M Tris, 0.15 M NaCl, pH 7.4) containing 0.06% H_2O_2 (for the peroxidase conjugate) or 0.01% 5-bromo-4-chloro-3-indolylphosphate predissolved in *N*,*N*-dimethyl formamide (Sigma) and 0.02% nitroblue tetrazolium (Sigma) predissolved in 70% *N*,*N*-dimethyl formamide and then diluted in 0.01 M $NaHCO_3$ and 0.001 M $MgCl_2$ (for the phosphatase conjugate). The conjugate-substrate control was performed by incubating the membrane with 1% skim milk and, after further washes, incubating the membrane with anti-mouse IgG-alkaline phosphatase conjugate and substrate.

ELISA was carried out using the immunoaffinity-purified fraction eluted from the MAb anti-VF-*T. crassiceps* column as the antigen. Each well of 96-well ELISA polystyrene high-binding plates (Costar Corning, Inc., Cambridge, Mass.) was coated with 100 μl of antigen (0.5 μg/ml) in 0.5 M carbonate-bicarbonate buffer (pH 9.6) for 18 h in a humidified chamber at 4°C. The wells were blocked for 1 h with 5% milk in PBS containing 0.05% Tween 20 and then incubated for 1 h with serum or CSF samples diluted 1:50 and 1:10, respectively. Peroxidase-conjugated goat anti-human IgG was added, and plates were incubated for 1 h. After each incubation step, the plates were washed using an automatic washer, with four cycles of saline (0.15 M NaCl) containing 0.05% Tween 20. *ortho*-Phenylenediamine (1 mg/ml) and H_2O_2 (1 μl/ml) diluted in 0.2 M citrate buffer (pH 5.0) were added (in the dark) as the chromogenic substrate, and plates were incubated for 20 min. The reactions were stopped by adding 100 μl of 2 M H_2SO_4. Color intensity was quantified using an ELISA plate reader (Diagnostics Pasteur, Strasburg-Schiltigheim, France) at 492 nm. All incubations were carried out at 37°C.

Statistical analysis. The cutoff values were determined using the receiver operating characteristic curve (Win Episcope 2.0). Comparisons in the median values among independent groups were performed using the nonparametric Kruskal-Wallis analysis of variance and Dunn's test. Analyses were made using Sigma Stat (Jandel Scientific Software). Significance was defined at the 5% level.

RESULTS

A total of eight MAbs (1 anti-ES-*T. crassiceps*, 1 anti-VF-*T. crassiceps*, 2 anti-VF-*T. solium*, and 4 anti-T-*T. solium*) obtained from four fusions cross-reacted with both *T. solium* and *T. crassiceps* antigens in an ELISA. In IB, these MAbs reacted with the 14-kDa protein from T-*T. solium* antigen and the 18- and 14-kDa proteins from VF-*T. crassiceps* antigen. All MAbs belong to the IgG isotype, one was IgG2b (anti-ES-*T. crassiceps*), and the others were IgG1 (Table 3).

From a total of 14.0 mg from VF-*T. crassiceps* antigen, 5.4 mg (38.5%) and 6.0 mg (43.0%) of purified fractions were obtained when anti-VF-*T. crassiceps* and anti-ES-*T. crassiceps* MAb columns, respectively, were used. These purified fractions presented relative molecular masses of 67, 18, and 14 kDa by SDS-PAGE (Fig. 1).

All MAbs (anti-ES-*T. crassiceps*, anti-VF-*T. crassiceps*, anti-VF-*T. solium*, and anti-T-*T. solium*) and serum or CSF samples from patients with NC recognized the 18- and 14-kDa proteins

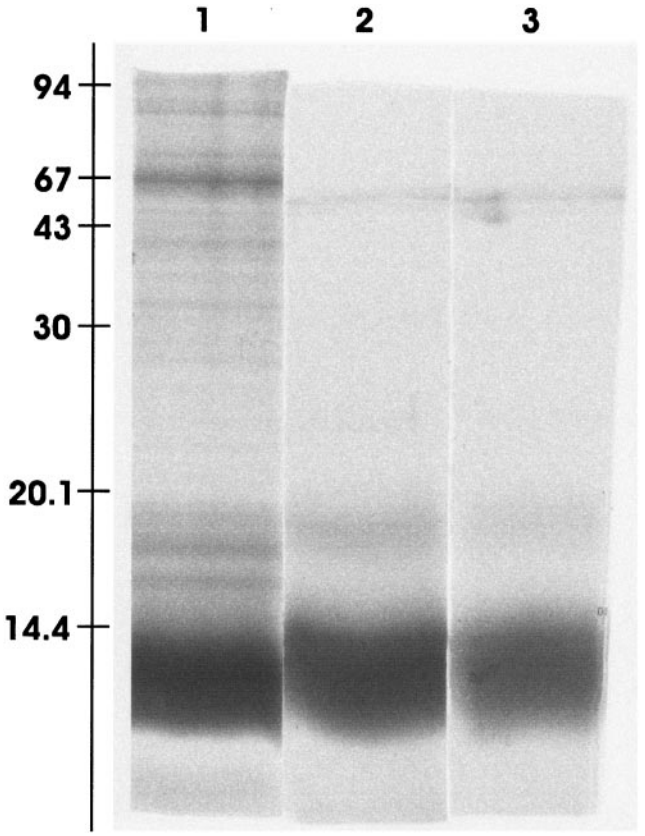

FIG. 1. Coomassie blue SDS-PAGE of VF-*T. crassiceps* antigen before purification (lane 1) and after purification by anti-ES-*T. crassiceps* (lane 2) and anti-VF-*T. crassiceps* (lane 3) MAbs. Molecular masses (in kDa) are shown at the left.

of VF-*T. crassiceps* purified antigen (Fig. 2). Although the 30-kDa protein was not observed by SDS-PAGE (Fig. 1), it was recognized by some (Fig. 2; see also Fig. 4). No reactivity was observed with the 67-kDa protein for any sample or MAb.

ELISA analysis of 23 CSF and 20 serum samples from patients with confirmed NC, using the *T. crassiceps* purified fraction as the antigen (ELISA-18/14), showed a sensitivity of 100%. All nine CSF samples with clinical findings and positive immunological tests were also positive by this assay (Fig. 3). The medians of absorbance to CSF and serum samples from patients with NC were 1.056 and 1.415, respectively.

ELISA-18/14 analysis of CSF samples from the 42 noninflammatory CSF controls showed a specificity of 100% (median of absorbance, 0.002). When 70 inflammatory CSF samples were included in the control group, the specificity was found to be 99.1% (confidence interval, 97.3% to 100%), and the median absorbance increased to 0.033 (Fig. 3). A single inflammatory CSF sample positive in the ELISA was from a patient with HIV-associated meningoencephalitis and showed strong reactivity in IB-18/14 (Fig. 4).

The specificity for the serum samples from 194 healthy individuals was 100%. When the 46 samples from patients with other parasitic diseases were included, the specificity was 98.7% (confidence interval, 97.3 to 100%). The median absorbances obtained for serum samples from healthy individuals,

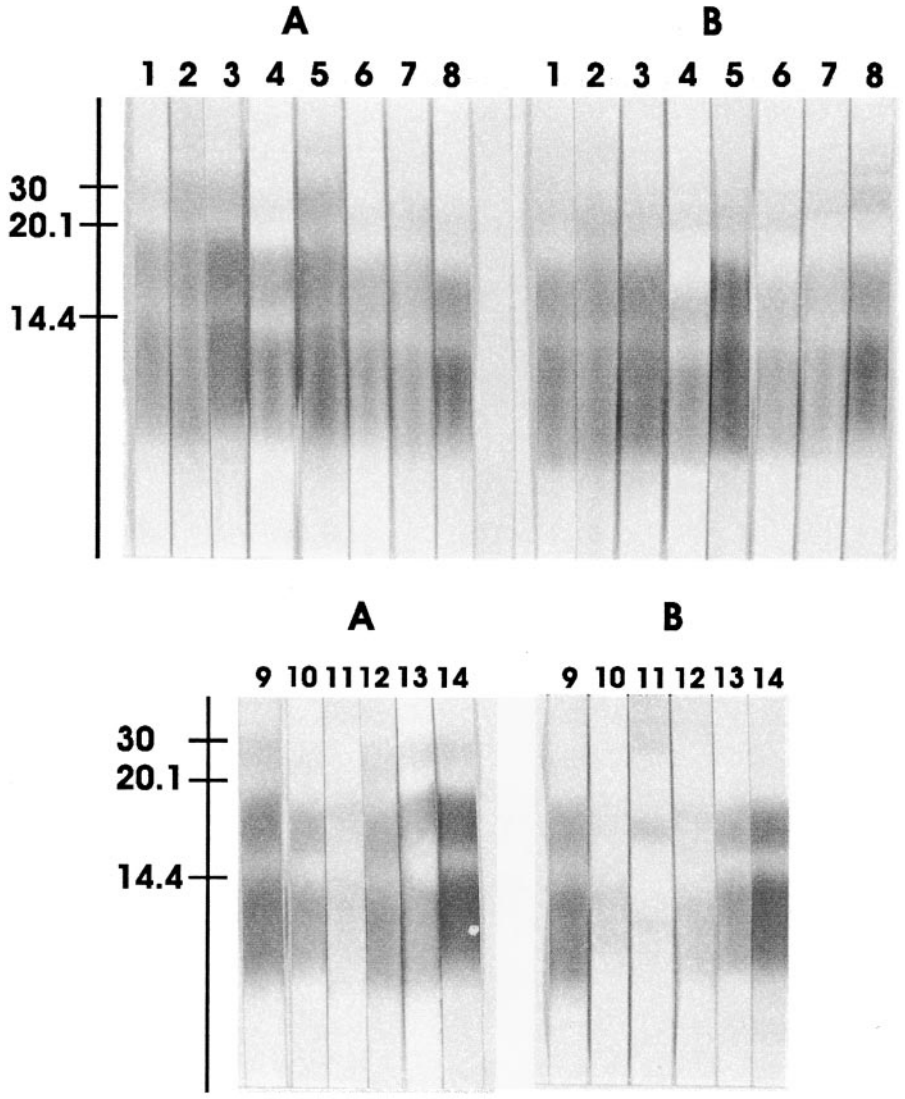

FIG. 2. Results of immunoblotting with both VF-*T. crassiceps* antigen (18- and 14-kDa proteins) purified with anti-ES-*T. crassiceps* (A) or anti-VF-*T. crassiceps* (B) MAbs. The reaction was performed using anti-ES-*T. crassiceps* MAb (lanes 1), anti-VF-*T. crassiceps* MAb (lanes 2), anti-VF-*T. solium* MAbs (clone A3 [lanes 3] and clone G12 [lanes 4]), and anti-T-*T. solium* MAbs (clone B11 [lanes 5], clone B4 [lanes 6], clone A6 [lanes 7], and clone E10 [lanes 8]) and also using serum (lanes 9 to 13) and CSF (lanes 14) samples from patients with NC. Molecular masses (in kDa) are shown at the left.

from patients with systemic parasitic diseases, and from patients with intestinal parasitic diseases were 0.130, 0.251, and 0.174, respectively. Three samples from patients with hydatidosis (ELISA-18/14 positive) were also positive in the ELISA with *T. solium* cysticerci antigens, and two of them were also positive by IB-18/14. Some CSF and serum samples with negative ELISA results were analyzed by IB-18/14 and found to be negative (Fig. 4).

DISCUSSION

In the present study, anti-*T. crassiceps* (anti-ES and anti-VF) MAbs selected by ELISA showed cross-reactivity with the *T. solium* antigen, and all anti-*T. solium* MAbs also presented cross-reactivity with the *T. crassiceps* antigen. This cross-reactivity confirms the presence of common antigenic determinants shared by the cysticerci, as reported before (2, 7, 13, 19, 27, 28). The reactivity of anti-*T. crassiceps* MAbs as well as of some polyclonal antibodies (CSF and serum samples from NC patients) toward the *T. crassiceps* antigen was abolished by treatment of the antigen with sodium periodate (data not shown), indicating that the chosen MAbs were specific for common immunodominant epitopes involved in the human immune response to cysticercus.

The reactivity of anti-*T. crassiceps* MAbs with *T. solium* and *T. crassiceps* antigenic fractions with relative molecular masses below 30 kDa and the specificity of these peptides in detecting antibodies in CSF and serum samples from patients with NC directed us to the purification of such peptides in the present study. Purified fractions of VF-*T. crassiceps* using MAb immunoaffinity chromatography corresponded to the native 18- and 14-kDa proteins. Immunoblot analyses showed that all anti-*T.*

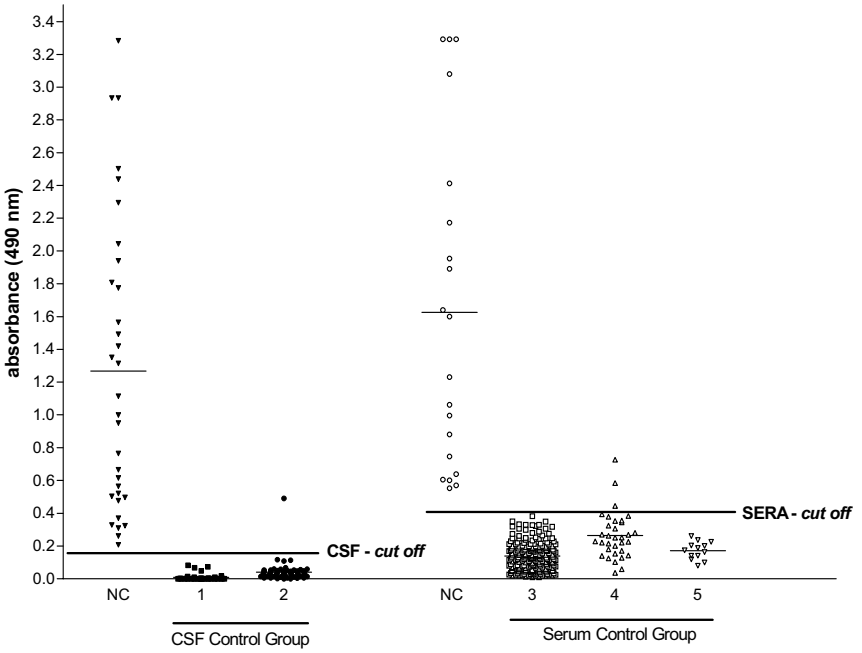

FIG. 3. Results of ELISA with VF-*T. crassiceps* antigen (18- and 14-kDa proteins) purified with anti-VF-*T. crassiceps* and CSF and serum samples from patients with NC. The CSF sample control group was composed of samples from patients with OND with noninflammatory (1) and inflammatory (2) CSF. The serum sample control group was composed of serum samples from apparently healthy individuals (3) and patients with systemic (4) and intestinal (5) parasitosis. The horizontal bars show the cutoff for testing CSF and serum samples.

crassiceps and anti-*T. solium* MAbs, as well as CSF and serum samples from patients with NC, identified both purified proteins. The importance of the 18- and 14-kDa proteins from *T. solium* was also demonstrated by others (8, 26). Greene et al. (8) showed that the purified fractions from lentil lectin-bound glycoprotein antigen contain multiple subunits that have potential as diagnostic antigens, particularly the 14-kDa subunit. The authors also suggest that, since the subunits are glycoproteins, differences between them could be due to the amino acid chain lengths and/or posttranslational modifications.

The 30-kDa protein of the antigen purified by immunoaffinity was only identified by IB, probably due to the higher sensitivity of the immunological methods compared with staining techniques used in SDS-PAGE, including silver staining (data not shown). A protein with a similar molecular mass was observed in the excretory/secretory antigens after in vitro culture of *T. crassiceps* cysticerci (7).

The 67-kDa protein identified by SDS-PAGE (Fig. 1) was not recognized by MAbs or antibodies from CSF and serum samples from patients with NC. Although no reactivity was

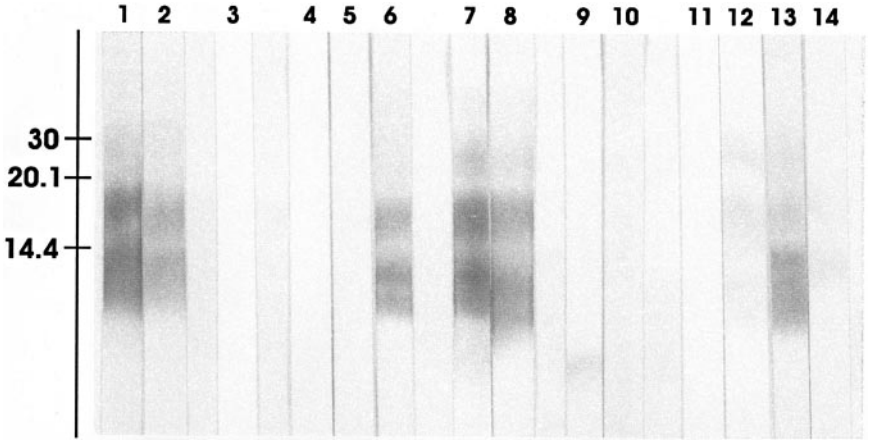

FIG. 4. Results of immunoblotting using VF-*T. crassiceps* antigen purified by anti-VF-*T. crassiceps* MAb and CSF (lanes 1 and 2) and serum (lanes 7 and 8) samples from patients with NC. The CSF sample control group was composed of samples of noninflammatory CSF (lanes 3 to 5) and inflammatory CSF from an HIV-associated meningoencephalitis patient (lane 6). The serum sample control group was composed of serum samples from apparently healthy individuals (lanes 9 and 10) and patients with hydatidosis (lanes 11 to 14). Molecular masses (in kDa) are shown at the left.

observed with the goat anti-mouse IgG-alkaline phosphatase conjugate (data not shown), the 67-kDa protein seems to be an artifact that appeared during the acidic desorption process and could be detectable only by SDS-PAGE, as reported by Kim et al. (12), using an anti-*T. solium* MAb.

The large amount and excellent fraction yield (38.5% and 43.0%) of 18- and 14-kDa purified peptides obtained from VF-*T. crassiceps* were reproducible in two batches using each of the MAbs (data not shown).

Using 18- and 14-kDa *T. crassiceps* purified proteins, we developed an immunodiagnostic method based on the ELISA system. The sensitivity of the test was 100% when 20 serum samples and 23 CSF samples from the NC group were assayed. For two patients with positive serum results, the paired CSF samples were negative for all antigens (VF-*T. crassiceps*, *T. solium*, and the 18- and 14-kDa proteins) by ELISA and IB. These two CSF samples were obtained after albendazole treatment of the patients, possibly during the calcification process. The calcification phase has been associated with a lower antibody concentration, especially in CSF, regardless of the antigen used (2, 13, 16, 29). Otherwise, five NC patients with ELISA-positive results for CSF samples presented negative results when paired serum samples were tested by ELISA with all antigens. However, very faint reactivity with the 30-, 18-, and 14-kDa proteins was observed by IB (data not shown). Three of the five negative serum samples were also negative by IB using VF-*T. crassiceps* and T-*T. solium* antigens, and the other two showed a very faint reactivity to the 14-kDa protein in the IB *T. crassiceps* and IB *T. solium* antigens (data not shown). The use of a larger quantity of purified antigen as well as another solid phase (e.g., dot blot) or the application of a lower cutoff would improve the sensitivity of ELISA-18/14 for these serum samples but may result in a lower specificity.

The ELISA-18/14 used on the control group showed 100% specificity for the noninflammatory CSF samples and 99.1% specificity when the inflammatory CSF samples were included. A significant difference was observed between absorbance results for CSF samples from the NC patients and CSF samples from the control group ($P < 0.0001$). In the control group, the single CSF sample that gave a positive result was from a patient with HIV-associated meningoencephalitis. This CSF sample was also strongly reactive for VF-*T. crassiceps* by IB and ELISA. We were unable to confirm the diagnosis of cysticercosis in this case because complementary clinical and neuroimaging data were not available. Detecting antibodies in CSF always seems to be specific unless immunoglobulins cross the blood-brain barrier during meningitis episodes, as reported with HIV infection (25).

The specificity for 194 serum samples in the control group (healthy individuals) was 100%, excluding serum samples from patients with hydatidosis. Three (10%) of the 30 serum samples from patients with hydatidosis were ELISA-18/14 positive (Fig. 3), and two of them were confirmed by IB-18/14 (Fig. 4). The specificity of the test including these samples was 98.7%, with a significant difference between the results for serum samples from patients with NC and the control group ($P < 0.0001$). Ishida et al. (11) reported 36 (77%) ELISA VF-*T. crassiceps*-positive results among 50 serum samples from patients with hydatidosis, but reactivity was confirmed in only 5 (10.9%) samples by IB using glycoproteins (18 to 14 kDa) from VF-*T. crassiceps* purified by affinity chromatography concanavalin A-Sepharose (20). Other authors have demonstrated the presence of common antigenic components of the cestodes *Taenia* and *Echinococcus* (6, 11). The 18- and 14-kDa proteins obtained in the present work were found to be specific for cysticercosis, and we are planning further studies to select those specific peptides from VF-*T. crassiceps* which are common to hydatid fluid from *E. granulosus*. It is possible that the conformation of the proteins should be highly important for either the immunogenicity or the antigenicity, and it is expected that more than one epitope is recognized by each individual, particularly in human cases, where immune responses are highly heterogeneous.

VF-*T. crassiceps* ELISA has shown a low specificity for serum samples, which indicates the need to perform confirmatory IB testing (1, 2). Bragazza et al. (1) described a 20% rate of false-positive results in 1,863 serum samples when a population from an area of cysticercosis endemicity was tested. By testing these samples by IB, the authors demonstrated that proteins of >30 kDa from VF-*T. crassiceps* were responsible for the unspecific reactions or cross-reactions with other parasitic infectious diseases. The cross-reactivities affecting the specificity index should be better evaluated in further clinical epidemiological studies using serum samples from different regions, including the most frequent heterologous parasitosis and other infections in each respective area.

In the present study, the use of 18- and 14-kDa *T. crassiceps* purified proteins in ELISA demonstrated high specificity and sensitivity for the diagnosis of human cysticercosis. Future analysis should validate its usefulness for screening seropositive individuals in immunoepidemiological studies with human and swine serum samples.

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