Taenia saginata Metacestode Antigenic Fractions without Affinity to Concanavalin A Are an Important Source of Specific Antigens for the Diagnosis of Human Neurocysticercosis

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Taenia saginata metacestode antigens have been constituted a useful alternative antigen for neurocysticercosis (NC) serodiagnosis, particularly due to an increasing difficulty to obtain Taenia solium homologous antigen. Cross-reactivity with Echinococcus granulosus infection occurs in homologous and heterologous antigens and could be avoided by using different purified methods. The present study evaluated antigen fractions obtained from saline extracts of T. saginata metacestodes purified by affinity chromatography with jacalin or concanavalin A (ConA) lectins to detect IgG antibodies by enzyme-linked immunosorbent assay (ELISA) and immunoblot analysis to diagnose human NC. Serum samples were collected from 142 individuals: 40 of them were diagnosed with NC, 62 presented Taenia sp. and other parasites, and 40 were apparently healthy individuals. The jacalin- and ConA-unbound fractions demonstrated sensitivity and specificity higher than those of bound fractions. Among unbound fractions, ConA demonstrated statistically higher sensitivity and specificity by ELISA (90% and 93.1%, respectively). By immunoblot assay, the 64- to 68-kDa component from the ConA-unbound fraction showed 100% sensitivity and specificity, making this component suitable for use as a specific antigen for diagnosis of NC. To our knowledge, this is the first report showing the relevance of using the unbound ConA fraction of T. saginata metacestodes to diagnose NC. In conclusion, the results obtained herein clearly demonstrate that antigenic fractions without affinity to ConA, obtained from T. saginata metacestodes, are an important source of specific peptides and are efficient in the diagnosis of NC when tested by immunoblot assay.

Taenia solium is a parasite whose larvae (metacestodes) may be located in the central nervous system of humans, causing neurocysticercosis (NC). Clinical manifestations are nonspecific and vary in severity, ranging from headaches, dizziness, and occasional seizures to a very severe neurological condition with intracranial hypertension or dementia (39, 40, 42). The diagnosis of NC is given by the combined analysis of clinical data and neuroimaging (computerized tomography and magnetic resonance imaging) as well as immunological and epidemiological data (10). From the neuroimaging findings of NC, only the presence of cystic lesions demonstrating the scolex should be considered pathognomonic (4). In many countries where T. solium is endemic, neuroimaging methods could be inaccessible and/or too expensive for the population at risk, often rural. Under these conditions, serology may provide a viable tool for diagnosis of the infection (6, 16, 18, 35).

As NC immunodiagnosis continues to be a challenge because of the increasing difficulty in obtaining parasites from naturally infected pigs for the preparation of T. solium homologous antigen (33), alternative antigens, including heterologous antigens from Taenia saginata, have also been used with satisfactory results to diagnose human NC (29, 31, 32). T. saginata cysticerci in the cystic stage may be obtained in considerable amounts, because the eating habits in many countries, including Brazil, are based mainly on cattle meat consumption. Although heterologous antigens from T. saginata metacestodes have been used with satisfactory results, there has been no research to test their purification with the purpose of selecting antigens present in this helminth that are able to react with antibodies induced by T. solium-infected individuals. Although our previous study has demonstrated high sensitivity and specificity with heterologous antigens but residual cross-reactivity between heterologous and homologous antigens (31), no research has been conducted so far to test the efficiency of purified fractions of saline antigen from T. saginata metacestodes for NC diagnosis.

Plant lectins are proteins capable of binding to carbohydrates on mammalian cells and membranes. Such binding can lead to a variety of effects, including cell agglutination, modification of enzymes or receptors on the cell surface, and stimulation of cell growth and various hormone-like responses. This property is also useful in purification by affinity chromatography (7). Lectins are proteins possessing at least one noncatalytic domain, which binds reversibly to a specific monosaccharide through hydrogen bonds, metal coordination, Van der Waals, and hydrophobic interactions (21). This sugar-binding property of the lectins also confers to these proteins the capacity to be isolated by affinity chromatography on matrices containing their specific ligand sugar. Affinity separation tech-
niques are based on biospecific molecular interactions. Therefore, they are extremely powerful tools for the isolation of valuable biological macromolecules (20).

Jacalin, the major protein from the jackfruit (Artocarpus integrifolia) seeds, is a tetrameric two-chain lectin with a molecular mass of 66 to 60 kDa (21). Concanavalin A (ConA) is a lectin protein originally extracted from the jack bean (Canavalia ensiformis). ConA is a tetrameric metalloprotein and binds molecules containing α-D-mannopyranosyl, α-D-gluco- pyranosyl, and sterically related residues. For a ConA reaction, the binding sugar requires the presence of the C-3, C-4, and C-5 hydroxyl groups. ConA can be used for the separation and purification of glycoproteins, polysaccharides, and glycolipids; detection of changes in the composition of carbohydrate-containing substances; isolation of cell surface glycoproteins from detergent-solubilized membranes; and separation of membrane vesicles (36).

The aim of this study was to purify the saline extracts of T. saginata metacestodes by jacalin and ConA Sepharose affinity chromatography and evaluate the extracts for the detection of immunoglobulin G (IgG) antibodies by ELISA and immunoblot assay in the diagnosis of human NC.

MATERIALS AND METHODS

Serum samples. This study was approved by the Federal University of Uberlândia Ethical Committee. Serum samples were collected from 142 subjects selected by the Laboratory of Clinical Analysis from Clinical Hospital groups 1 and 2) and the Laboratory of Parasitology (group 3) of the Federal University of Uberlândia in the state of Minas Gerais, Brazil. Group 1 comprised 40 patients who had been diagnosed with definitive NC, based on their clinical presentation, and the evidence of household contact with T. solium in at least two patients; (iii) for immunological diagnosis, cerebrospinal fluid and/or serum samples were positive in an ELISA for the detection of IgG anticysticercal antibodies; (iv) for cerebral computerized tomography findings, all patients presented evidence of parasite neuroimaging, with 8 (20%) presenting vesicular, 15 (37.5%) vesicular/calculated, and 17 (42.5%) calculated metacestodes. Group 2 comprised 62 patients presenting other parasthesias: Ascaris lumbricoides (6 patients), Echinococcus granulosus (10 patients), Enterobius vermicularis (6 patients), Giardia lamblia (4 patients), hookworm (6 patients), Hymenolepis nana (4 patients), Schistosoma mansoni (10 patients), Strongyloides stercoralis (4 patients), Taenia sp. (10 patients), and Trichuris trichiura (2 patients). Group 3 comprised 40 apparently healthy volunteers, based on their clinical presentation. Although they came from an area where cysticercosis is endemic, all volunteers from this group did not present evidence of household contact with T. solium infection or a history of taeniasis or cysticercosis. In addition, these individuals had three fecal samples that tested negatively by the parasitological methods of Lutz (25) and Baermann (1).

Parasites and antigens. Fresh T. saginata metacestodes were collected from naturally infected bovine muscle and were carefully dissected from the host tissues, washed repeatedly, and stored at −20°C. A saline extract was prepared as described previously by Oliveira et al. (31). T. SAGINATA FOR HUMAN NEUROCYSTICERCOSIS DIAGNOSIS

Isolation of jacalin-reactive components from total extract of T. saginata metacestodes. The isolation of ConA-reactive components was carried out according to the method of Hermanson et al. (17), with some modifications. Glycosylated protein from a saline extract of T. saginata metacestodes (12 mg total protein) was isolated by affinity chromatography using 5 ml jacalin immobilized on cross-linked 4% bead agarose (Sigma-Aldrich, Chemical Co., St. Louis, MO) previously equilibrated with phosphate-buffered saline (PBS). The flow of the column was stopped, and the column incubated overnight under continuous slow agitation at 4°C. The unbound material was washed with PBS, and jacalin-bound components were eluted with 0.4 M α-galactose (Sigma) at a flow rate of 0.5 ml/min, monitored by absorbance at 280 nm, and collected in 2-ml fractions. Jacalin-bound and jacalin-unbound fractions were independently concentrated using a stirred ultrafiltration cell (Amicon, YM-10; W. R. Grace & Co.), and the jacalin-bound fraction was dialyzed against PBS. The protein of each antigen preparation was determined (24).

Isolation of ConA-reactive components from total extract of T. saginata metacestodes. The isolation of ConA-reactive components was carried out according to the method of Hermanson et al. (17), with some modifications. Glycosylated protein from a saline extract of T. saginata metacestodes (12 mg total protein) was isolated by affinity chromatography using 5 ml ConA immobilized on cross-linked 4% bead agarose (Sigma) previously equilibrated with PBS. The flow of the column was stopped, and the column incubated overnight under continuous slow agitation at 4°C. The unbound material was washed with PBS, and ConA-bound and ConA-unbound fractions were independently concentrated using a stirred ultrafiltration cell (Amicon), and the ConA-bound fraction was dialyzed against PBS. The protein of each antigen preparation was determined (24).

SDS-PAGE. Antigens were diluted (vol/vol) in sample buffer, and after boiling at 98°C for three min, all antigen preparations and molecular weight markers (Sigma) were submitted to SDS-PAGE conditions were as described previously by Oliveira et al. (31). The reaction index (RI) was calculated (33). The data were submitted to two-graph receiver operating characteristic (TC-ROC) analysis, which is a plot of the test sensitivity (Se) and specificity (Sp) against the threshold (cutof) value, assuming the latter to be an independent variable. The calculation was based on a logarithm of the migration of a set of molecular weight standards included in each gel.

Immunossays. (i) ELISA. An ELISA using a saline extract and fractions was carried out according to the method of Oliveira et al. (31). Briefly, polystryrene microplates (Interlab, Brazil) were coated with each extract at concentrations of 10 µg/ml in 0.06 M carbonate bicarbonate buffer, pH 9.6. These preparations were incubated overnight at 4°C in a final volume of 50 µl/well. Microplates were washed three times, 5 min each time, with PBS containing 0.05% Tween 20 (PBS-T), and the serum samples diluted 1:200 in PBS-T were added and incubated for 45 min at 37°C. After the washing procedure described above, the enzyme conjugate (peroxidase-goat anti-human IgG, Fc specific; Sigma) was added in a 1:1,000 dilution in PBS-T for all antigens and incubated for 45 min at 37°C. The assay was developed after the washing procedure by adding the enzymatic substrate consisting of H2O2 and orthophenylendiamidine (OPD) in 0.1 M citrate phosphate Na2HPO4 buffer, pH 5.5, for 15 min, which was followed by 25 µl well of H2SO4 (2 N) to stop the reaction. Optical densities (OD) were determined at 492 nm in an ELISA reader (Tp Reader; Thermoplate, China). The cutoff values were established using the mean OD of three nonreactive serum samples plus two standard deviations. The reactivity index (RI) was determined (24). The cutoff was calculated for each antigen and was obtained as the intersection point of the two graphs (13). All the samples showing an RI value of >1.1 were considered positive for jacalin- and ConA-unbound fractions.

(ii) Immunoblot assay. The immunoblot assay was performed according to the method of Oliveira et al. (31). Antigen preparations were subjected to electrophoresis and transferred to nitrocellulose membranes (0.45 µm; Sigma) by using a transfer apparatus (Omniphor, England). Nitrocellulose strips containing antigen preparations were blocked with 5% nonfat milk in PBS-T (PBS-TM) for 2 h at room temperature and incubated overnight with serum samples diluted 1:50 in 1% PBS-TM at 4°C. After six washes with 1% PBS-TM, the strips were incubated for 2 h at room temperature with peroxidase-labeled goat IgG anti-human IgG conjugate, whole molecule (Sigma), diluted 1:1,500 in 1% PBS-TM. The strips were washed in PBS and developed for 3 min in substrate solution containing hydrogen peroxide and 3,3-diaminobenzidine tetrahydrochloride (DAB-Sigma) in PBS. The reaction was stopped by washing the strips with distilled water, and positive reactions were determined by the appearance of clearly defined bands. The relative molecular masses of the recognized bands were determined by comparison with molecular markers (Sigma) stained with silver. The data were submitted to two-graph receiver operating characteristic (TC-ROC) analysis, which is a plot of the test sensitivity (Se) and specificity (Sp) against the threshold (cutof) value, assuming the latter to be an independent variable. The calculation was based on a logarithm of the migration of a set of molecular weight standards included in each gel.

Data analysis. The geometric means (gm) of the RI values were calculated for each group and extract. Differences between two proportions were calculated by a binomial test (two proportions). A null hypothesis was rejected when the P value was <0.05. Sensitivity, specificity, and diagnostic efficiency (accuracy) were calculated (2), and the Youden index (YI) was determined (44).
RESULTS

As demonstrated in Fig. 1, all samples were tested by ELISA using three antigen preparations. It was observed that 36 (90%) of the serum samples from group 1 were positive for saline extract antigen, 24 (60%) for jacalin-unbound antigen, and 36 (90%) for ConA-unbound antigen. The difference between the jacalin-unbound and the other antigens was statistically significant ($P < 0.05$).

For group 2, 16 (25.8%) of the serum samples were positive for saline antigen, 16 (25.8%) were positive for jacalin-unbound antigen, and 7 (11.3%) were positive for ConA-unbound antigen. The cross-reactivity in the ELISA for group 2 was due predominantly to *E. granulosus* infection, although the reactivity against this parasite became lower using ConA-unbound antigen ($P < 0.05$). The profile of cross-reactivity using saline antigen was *A. lumbricoides*, 2/6; *E. granulosus*, 10/10; *H. nana*, 2/4; and *S. stercoralis*, 2/4. Using jacalin-unbound antigen, the profile was *A. lumbricoides*, 3/6; *E. granulosus*, 9/10; *H. nana*, 2/4; and *S. stercoralis*, 2/4. The best profile from the ELISA was obtained using ConA-unbound antigen, where *E. granulosus* was 6/10 and *S. stercoralis* 1/4.

All serum samples from group 3 were negative when using ConA-unbound antigen, while 3 (7.5%) samples from this group were positive for saline antigen and 4 (10%) were positive for jacalin-unbound antigen.

Therefore, considering the results obtained for group 2 and 3 serum samples, ELISA specificity was 81.4% for saline antigen, 80.4% for jacalin-unbound antigen, and 93.1% for ConA-unbound antigen. These data resulted in Youden index values of 0.7, 0.4, and 0.8 for saline extract, jacalin-unbound, and ConA-unbound antigens, respectively. The diagnostic efficiencies were determined as 83.8%, 74.6%, and 92.3% for saline extract, jacalin-unbound, and ConA-unbound antigens, respectively.

Figure 2 shows the electrophoretic profiles obtained for saline extract and their fractions as well as the results of the

![Figure 1](http://cvi.asm.org/) Detection of anti-*T. solium* metacestode IgG antibodies in serum samples from patients with a definitive diagnosis of NC (group 1; $n = 40$), individuals infected with *Taenia* sp. and other parasites (group 2; $n = 62$), and apparently healthy individuals (group 3; $n = 40$) by ELISA using saline extract, jacalin-unbound fraction, and ConA-unbound fraction antigens from *T. saginata* metacestodes. The horizontal bar indicates the cutoff (RI = 1.1). gm, geometric means.

![Figure 2](http://cvi.asm.org/) Electrophoretic profile (left) of saline extract (lane A), jacalin-bound (lane B), jacalin-unbound (lane C) ConA-bound (lane D), and ConA-unbound (lane E) fractions in SDS-PAGE at 12% by silver staining. Immunoblot assay (right) of representative samples of patients with a definitive diagnose of NC (lane 1), individuals infected with *E. granulosus* (lane 2) or *A. lumbricoides* (lane 3), and apparently healthy individuals (lane 4) using saline extract (blot A), jacalin-unbound (blot C) and ConA-unbound (blot E) fractions. The same serum samples were tested with all three antigen preparations. Arrows indicate reactivity to 64- to 68-kDa component.
immunoblot assay, SDS-PAGE showed differences between bound and unbound antigens, suggesting a different profile in the protein recognition. The immunoblot assay, which was performed on all ELISA-reactive serum samples, showed the presence of a wide range of molecular markers, varying from 116 to 12 to 14 kDa when using saline antigen. The jacalin-unbound fraction revealed 110- to 64- to 68-, and 39- to 42-kDa molecular-mass markers, whereas the ConA-unbound fraction revealed only 110- and 64- to 68-kDa molecular-mass markers. The lack of specificity for the jacalin- and ConA-bound fractions by the immunoblot assay was due mainly to reactivity with components presenting high molecular weights. Also, both fractions did not present reactivity with 64- to 68-kDa molecular-mass markers, which was considered a critical point in terms of the sensitivity of the assay to detect specific antibodies in neurocysticercosis patients.

The use of the ConA-unbound fraction demonstrated a strong reactivity (100%) of 64- to 68-kDa protein in patients diagnosed with definitive NC (Fig. 3A). On the other hand, this protein was not recognized with any patients infected with Taenia sp. and other parasites (Fig. 3B). No protein was recognized with healthy individuals using the ConA-unbound fraction (Fig. 3C). The comparison of the results obtained among all three antigen preparations revealed that the ConA-unbound fraction demonstrated the highest specificity ($P < 0.05$).

**DISCUSSION**

In the present study, we described a purification process of the saline extract from *T. saginata* metacestodes utilizing jacalin and ConA Sepharose affinity chromatography in the diagnosis of human NC, for which the ConA-unbound fraction obtained provided evidence of being more specific than the other antigen preparations.

Lectins are multivalent carbohydrate-binding proteins that recognize diverse sugar structures with high specificity (20). ConA was the first legume lectin recognized as a mannospecific protein (41). The major carbohydrate-binding proteins from various pathogens are present in the plasma membrane, and different studies described the importance of surface antigen in the sensitivity and specificity of immunodiagnosis, including for NC (5, 9, 12). In the present study, the purification by Sepharose affinity chromatography resulted in jacalin- and ConA-unbound and jacalin- and ConA-bound fractions. Interestingly, a distinct electrophoretic profile was observed for jacalin- and ConA-unbound fractions, while jacalin- and ConA-bound ones showed few similar components, considering their molecular weights.

A recent study comparing the saline extract from *T. solium* metacestodes and from *T. saginata* demonstrated a statistically significant difference in the immunodominant band recognition (31). The 24- and 56-kDa bands were the most frequent in the reaction using saline extract from *T. solium* metacestodes compared to those of 39 to 42 kDa. The 64- to 68-kDa bands were the most frequent when using saline extract from *T. saginata* metacestodes. The 47- to 52-kDa bands were recognized by both antigens, without differences in the frequencies. This variation can be related to the presence of different antigenic epitopes between both helminth species, justifying the lack of the 50-kDa immunodominant protein that has been reported in many papers and demonstrating high levels of sensitivity and specificity (15, 27, 43).

In the present study, cross-reactivity was observed among serum samples from individuals infected by *H. nana* when using saline extract and jacalin-unbound fractions and tested by ELISA. This observation may be related to the presence of common antigenic components between these species due to the phylogenetic proximity between *Taenia* sp. and *Hymenolopsis* sp. (*Taeniidae* family), as previously described (28, 37). Cross-reactivity in *S. stercoralis* infections was also observed in the present study when the serum samples were tested by ELISA using a saline extract or jacalin-unbound fraction. Considering that there is minimal phylogenetic proximity between *Taenia* sp. and *Strongyloides* sp., these results may be related to the presence of infections with both species (*T. solium* and *S. stercoralis*) in these individuals, since human cysticercosis is considered endemic (30, 38) and *S. stercoralis* hyperendemic (26) in the city of Uberlândia within the Triângulo Mineiro region.

The major specificity problem using homologous or heterologous antigen in NC diagnosis is the *E. granulosus* infection. Accordingly, previous studies have demonstrated low specificity when serum samples of individuals infected by *E. granulosus* were tested by indirect immunofluorescence, the ELISA, and immunoblotting using antigens from *T. solium* metacestodes and *T. saginata* metacestodes (11, 19, 31). The presence of common stage-specific antigenic components and homologous surface antigens expressing genes from *Taenia* sp. and *Echinococcus* sp. was thus demonstrated (12, 15). These antigens are responsible for the cross-reactivity between both parasites, even though previous studies using whole-metacestode extracts have demonstrated that both the ELISA and immunoblotting may be considered useful tools for epidemiological screening of NC (30, 34, 38). Therefore, as cross-reactivity between *T. solium* and *E. granulosus* occurs in screening tests such as the ELISA, additional assays are required to validate the results, preferentially by using alternative assay designs with purified and more specific antigens.

In the present study, the fractions obtained by ConA Sepharose affinity chromatography proved to be more specific than the other fractions, as no samples from healthy individuals were reactive, but 60% of the serum samples from patients with *E. granulosus* showed reactivity, indicating that the diagnostic efficiency with these fractions by the ELISA needs improvement. In contrast, the immunoblot assay using the ConA-unbound fraction demonstrated 100% sensitivity and specificity when defining the 64- to 68-kDa immunodominant protein to differentiate patients from group 1 and group 2 or healthy individuals (group 3). A previous study identified seven immunodominant proteins (135, 100, 86, 64, 39, 35, and 24 kDa) observed with individuals infected with *E. granulosus* by using immunoblotting with vesicle liquid from *T. solium* metacestodes (22). In the present study, however, the immunodominant protein selected to define the NC diagnosis was 64 to 68 kDa and obtained from the ConA-unbound fraction from *T. saginata*, which may be considered different from the cross-reactive components described by those investigators (22). In addition, our results are in agreement with previous studies that found low specificity when the immunodominant proteins...
FIG. 3. Frequency of immunodominant proteins (kDa) recognized by immunoblotting when using saline extract, jacalin-unbound, and ConA-unbound fractions probed against serum samples from 40 patients with NC (A), 62 individuals with other parasites (B), and 40 healthy individuals (C).
have molecular masses higher than 70 kDa or belong to the 8-kDa group of molecular markers (14, 43).

In conclusion, the results obtained herein clearly demonstrate that antigenic fractions without affinity to ConA, obtained from *T. saginata* metacestodes, are an important source of specific peptides and are efficient in the diagnosis of NC when tested by immunoblotting. To our knowledge, this is the first report showing the relevance of using the unbound ConA fraction of *T. saginata* metacestodes to diagnose NC.

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