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DISCRIMINATION BETWEEN ACTIVE AND INACTIVE NEUROCYSTICERCOSIS BY METACESTODE EXCRETORY/SECRETORY ANTIGENS OF *TAENIA SOLIUM* IN AN ENZYME-LINKED IMMUNOSORBENT ASSAY

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Abstract. To detect IgG antibodies to *Taenia solium,* a controlled double-blind study was conducted using 91 coded cerebrospinal fluid samples from patients with neurocysticercosis (NCC) and other neurologic disorders. Samples were tested in an enzyme-linked immunosorbent assay (ELISA) using metacestode excretion/secretion antigens. The results were correlated with data from medical records on the diagnosis of NCC (based on computed tomography and magnetic resonance imaging criteria) and other neurologic disorders. The ELISA results were positive in 22 of the 24 cases with active NCC. In contrast, six cases with calcified cysts (inactive NCC), as well as one case in a transitional stage, were negative. One case with a calcified granuloma and another with a granuloma plus calcifications (classified as inactive NCC) had positive results. The remaining negative results corresponded to other neurologic disorders (58 cases). The results of the ELISA showed a significant difference between active and inactive NCC ($P = 0.0034$).

INTRODUCTION

Neurocysticercosis (NCC) is the generic term used to denote the parasitic disease of the human nervous system caused by the metacestode of *Taenia solium*. Classifications of NCC have been proposed according to the biologic stage of the parasite, its morphology, and the degree of secondary inflammation. Magnetic resonance imaging (MRI) and computed tomography (CT) scans have been very useful in developing the following classification: active NCC, when metacestodes are alive; transitional NCC, when metacestodes are in a degenerative state; inactive NCC, when metacestodes are calcified; and mixed NCC, when two or three of the previous types coexist.[1–3]

The pleomorphism of the clinical manifestations in human NCC makes it difficult to establish a presumptive diagnosis. Signs and symptoms appear after an asymptomatic period, typically 4–5 years, but this can be up to 30 years.[4] Since it has been accepted that the abrupt onset of clinical signs in human NCC coincides with intense pathologic reactions to degenerating metacestodes in the brain,[5] the incubation period may approximate the estimated life span of the metacestodes.[6] Thus, the disease may result primarily from the host inflammatory response to dying parasites.[7]

Many efforts have been made to use homologous antigens,[8–14] *T. crassiceps* antigens and synthetic peptides,[15–17] a phage display peptide library,[18] and recombinant *T. solium* antigens in the immunodiagnosis of cysticercosis.[19–22]

Host-parasite interaction is poorly understood in NCC. Studies of brain metacestodes from cysticercotic patients have shown a predominance of a cell-mediated response and the presence of interleukin-12 (IL-12).[23,24] It has also been reported that IL-5 and IL-10 predominated in the cerebrospinal fluid (CSF) of patients with active NCC.[25] Cysteine, aspartic, and metalloprotease activities have been characterized in excretory/secretory (E/S) antigens from *T. solium* metacestodes.[26,27] Live metacestodes may release such proteases for parasite nutrition, and they may also induce a humoral immune response in the host. The linkage between live metacestodes and the induced humoral response prompted us to test for IgG antibodies against metacestode E/S antigens of *T. solium.* An enzyme-linked immunosorbent assay (ELISA) and an enzyme-linked immunoelectrotransfer blot (EITB) assay using metacestode E/S antigens were performed on samples of CSF from patients with neurologic disorders. The ELISA results showed a significant difference between active and inactive NCC ($P = 0.0034$).

MATERIALS AND METHODS

Metacestode E/S antigens. Cysticercotic pigs (10–12 months old) were acquired from an endemic area of porcine cysticercosis.[28,29] After the pigs were humanely killed, metacestodes from each pig were obtained as described.[30] Briefly, 20 metacestodes dissected from muscle tissues were incubated in 20 ml of RPMI 1640 medium (Sigma, St. Louis, MO) containing glucose (1 μg/ml) and trypsin (0.5 μg/ml) at 37°C in an atmosphere of 5% CO_2 for four hours. In cases that showed scolex evagination greater than 90% and intense motility of scolices, additional metacestodes were dissected, washed extensively with sterile phosphate-buffered saline (PBS) containing penicillin (500 units) and streptomycin (500 μg/ml). Metacestode E/S antigens were obtained as described[27] with modifications. Metacestodes (700/culture) were incubated in 10 mL of 9 mM Tris buffer, pH 7.2, containing 1 mM EDTA and 2×10^{-5} M E-64 (inhibitors for metalloproteinases and cysteine proteases, respectively) at 37°C in an atmosphere of 5% CO_2 for three hours. Culture fluids were centrifuged at $9{,}000 \times g$ for 20 min, filtered through 0.45-μm Millipore (Bedford, MA) membrane, lyophilized, and stored at −70°C.

Protein determination. Determination of protein concentration in metacestode E/S antigens was carried out according to the method of Bradford.[31]

Cerebrospinal fluid samples. Cerebrospinal fluid samples from 91 patients with neurologic disorders were provided by the Sera and CSF Bank of the National Institute of Neurology and Neurosurgery of Mexico. Samples were collected from 1982 to 1994 and kept at −20°C. Codified samples were tested in a double-blind controlled study.

Detection of IgG antibody by ELISA. Detection of antibody by ELISA was performed according to the procedure of Diaz and others[9] with modifications. Metacestode E/S antigens at a concentration of 3 μg of protein/ml were suspended in carbonate buffer (pH 9.6). Antigen (100 μl) was placed in wells of microtiter plates in triplicate (Costar, NY). Plates were incubated overnight at 4°C with PBS containing 3% non fat skimmed milk and 0.05% Tween 20, followed by incubation at 37°C for one hour. Cerebrospinal fluid was diluted 1:10, 100 μl was added to the plate, and the plate was incubated at 37°C for 30 min. The plates were then washed four times with PBS-0.05% Tween 20 and 100 μl of peroxidase-conjugated goat anti-human antibody (1:3,000 dilution) were added. The plates were incubated at 37°C for 30 minutes, washed four times with PBS-Tween, and 200 μl of freshly prepared substrate (0.4 mg of *o*-phenylendiamine/ml plus 0.06% H_2O_2 in citrate-phosphate buffer, pH 5) were added. The reaction was allowed to proceed for 5 minutes at room temperature and was stopped by the addition of 20 μl of 0.1 M sulfuric acid. The optical density (OD) was measured at 492 nm in a spectrophotometer (Labsystems, Stockholm, Sweden).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was carried out according to the method of Laemmli[32] in a Mini-Protean II cell (Bio-Rad, Hercules, CA). Samples of native and metacestode E/S antigens preincubated with E-64 were analyzed. The gels were stained with a silver stain (Sigma). Molecular weight was determined using prestained SDS-PAGE standards (Bio-Rad).

Enzyme-linked immunoelectrotranfer blot assay. To detect the antigens that were recognized by the CSFs, an EITB assay was carried out on a nitrocellulose sheet in a Mini-Transblot cell (Bio-Rad). After metacestode E/S antigens were electrophoretically transferred to the sheet, it was removed and washed four times with PBS-Tween for (15 minutes/wash). It was then cut into strips 0.4 cm wide and exposed to CSF samples diluted (1:5) with PBS-Tween on multi-channel plaques at room temperature for two hours with shaking. The strips were washed three times with PBS-Tween for (10 minutes/wash) and incubated with 1 ml of biotinylated protein A (1:400 dilution) at room temperature for one hour. The strips were then washed with PBS-Tween, incubated with 1 ml of streptavidin (diluted 1:400) at room temperature for 30 minutes, and washed twice with PBS-Tween (5 minutes/wash) and with PBS alone. The reaction was revealed with a substrate solution containing 0.005% 3-3′ diaminobenzidine (Sigma) in PBS, pH 7.2, plus 3% H_2O_2 and stopped with deionized water.

Analysis of medical records. Data on the biologic stage of the parasites (live, transitional stage, and calcified cysts) were obtained from CT and MRI reports. These data were correlated with the results of the ELISA.

Statistical analysis. A positive ELISA result was defined as four standard deviations above the mean OD (0.105 ± 0.037, range = 0.034–0.169) of 58 CSF negative control samples from patients with neurologic disorders other than NCC. This value was calculated to be an OD of 0.185. Sensitivity was calculated as the number of ELISA-positive cases/total number of cysticercotic patients. Data were expressed as mean ± standard error of the OD values. Differences between active and inactive NCC were analyzed by Student's *t*-test. *P* values < 0.05 were considered significant.

RESULTS

Results of the ELISA and their correlation with CT and/or MRI findings. The 91 CSF samples were tested by ELISA using metacestode E/S antigens. The ELISA values greater than the cut-off value (OD = 0.185) were considered positive (Figure 1).

The CT and/or MRI findings showed that 33 patients had a diagnosis of NCC (23 women and 10 men, age range = 18–88 years). The remaining patients had other neurologic disorders (n = 58). Cases were classified into two categories: active and inactive NCC. The active NCC category included patients with ELISA values greater than 0.185 and a diagnosis of NCC by CT and/or MRI (n = 22). Two cases with viable plus calcified cysts with negative ELISA results were included in this category to calculate the *P* value. Cases were grouped according to the following characteristics: 1) one or more viable cysts (data on the precise number of multiple lesions were not found in medical records) or 2) one or more viable cysts plus cysts either in a transitional, racemose, or calcified stage. The inactive NCC category included six patients with calcified cysts, one case with a colloidal cyst, and another patient with one granuloma plus calcifications. The CSF from patients with calcified and colloidal cysts exhibited negative ELISA values (< 0.185). The difference between these two categories was statistically significant ($P = 0.003$; Figure 2).

The CSF samples from two patients with other neurologic disorders and without evidence of cysticercotic lesions by CT and MRI showed false-positive results. One of them (OD = 0.280) was from a 49-year-old patient with meningitis caused by *Mycobacterium tuberculosis.* The other sample (OD = 0.203) was from a 52-year-old patient with acute bacterial meningitis and positive serologic results for human immunodeficiency virus (HIV) (Figure 1).

To better define the relationship between OD values and

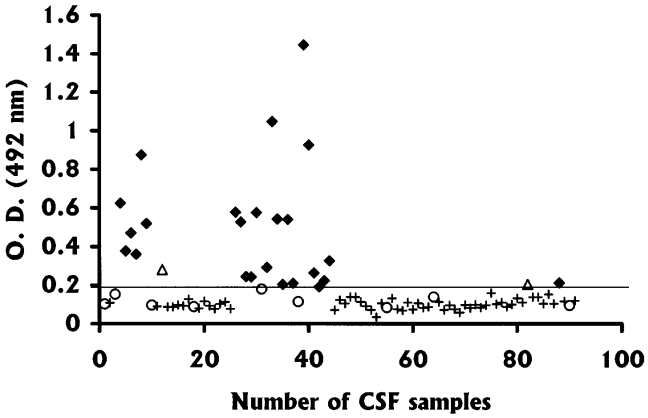

FIGURE 1. Enzyme-linked immunosorbent assay (ELISA) optical density (O.D.) values of 91 coded cerebrospinal fluid (CSF) samples from patients at the National Institute of Neurology in Mexico. The ELISA was conducted using metacestode excretory/secretory antigens. ♦ = Positive cases diagnosed as active neurocysticercosis (NCC) by computed tomography (CT) or/and magnetic resonance imaging (MRI); Δ = positive cases diagnosed as neurologic disorders other than NCC; O = negative cases diagnosed as inactive NCC by CT or/and MRI; + = negative cases diagnosed as disorders other than NCC. The **horizontal line** is the cut-off ELISA value (OD = 0.185, mean + 4 SD of 58 cases diagnosed as other disorders other than NCC).

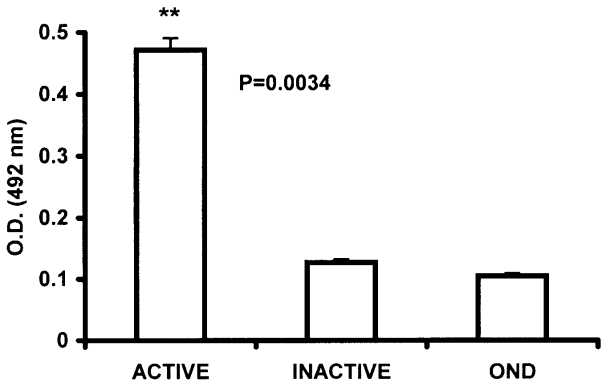

FIGURE 2. Histogram of enzyme-linked immunosorbent assay (ELISA) values of cases with active and inactive neurocysticercosis (NCC). Active = cases of NCC with viable cyst(s) with or without transitional or calcified cysts (n = 24); Inactive = cases of NCC with calcified cysts, one case with a colloidal cyst, and one case with a granuloma plus calcifications (n = 8); OND = cases of other neurologic disorders. Bars show the mean ± SD O.D. = optical density. $^{**}P = 0.0034$, by Student's *t*-test.

the biologic stage of the parasites, cases were grouped as follows. The highest positive OD values (0.589 ± 0.1449) corresponded to cases with one viable cyst (n = 6; $P = 0.008$). Intermediate positive OD values (0.537 ± 0.065) corresponded to cases with one or more viable cysts plus one or more transitional cysts (n = 7; $P = 0.0001$). The lowest positive OD values (0.398 ± 0.157) corresponded to cases with one viable cyst plus one or several calcified cysts (n = 8; $P = 0.134$, not significant). The ELISA values of six cases with a single lesion and characteristics different from those described above are also depicted (Table 1). Negative ELISA values from cases with calcified cysts were compared with ELISA data reported in medical records. The comparison showed that cases with calcified cysts had positive ELISA values when metacestode extract antigens were used.

SDS-PAGE. Electrophoresis showed differences between metacestode E/S antigens treated with E-64 and those not treated (Figure 3C, lanes 3 and 2, respectively).

Immunoblot assays. Results of the EITB assay showed a good correlation with those of the ELISA. The CSF samples

TABLE 1

Correlation between enzyme-linked immunosorbent assay values (mean ± SEM) and the biological stage of cysts (data obtained from computed tomography/magnetic resonance imaging scans reports)

Parasite stage	Number of cases	OD values (mean ± SEM)	*P**
Viable (single)	6	0.589 ± 0.1449	0.0075
Viable (multiple)	1	0.263	
Colloidal	1	0.179	
Viable(s) plus transitional	7	0.537 ± 0.0651	0.0001
Viable(s) plus calcifications	8	0.398 ± 0.1568	0.1343
Viable plus racemose	1	0.242	
Calcified granuloma	1	0.519†	
Granuloma plus calcifications	1	0.211	
Calcified cysts	6	0.105–0.0078	
Racemose	1	0.326	
Total	33		

* *P* values of calcified cysts.
† Recognized by 4 antigens in the blotting assay.

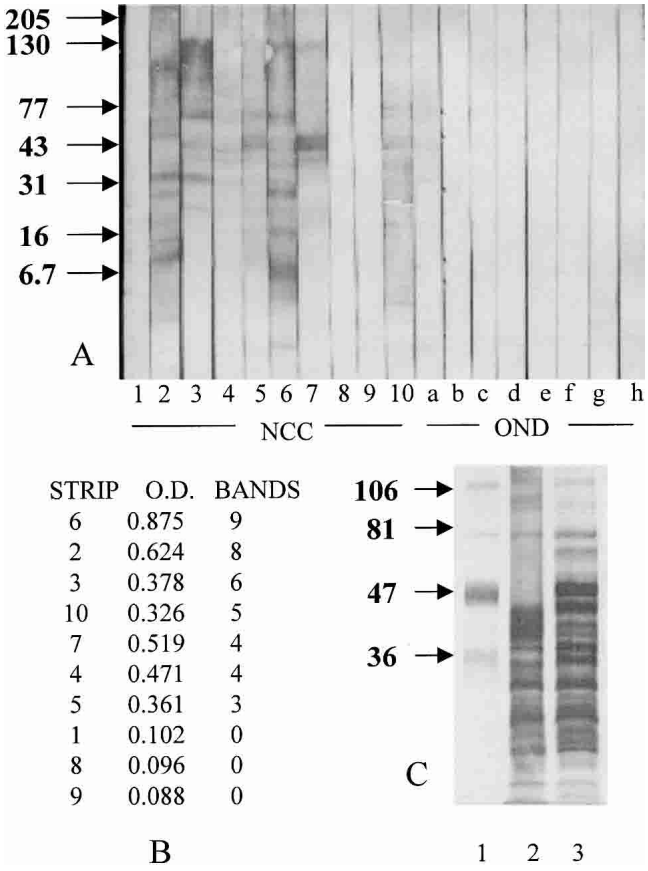

FIGURE 3. **A**, Blotting results of 10 cerebrospinal fluid samples from patients with different forms of active and inactive neurocysticercosis (NCC) (lanes 1–10) and eight samples from patients with other neurologic disorders (OND) (lanes a–h). Lane 7 corresponds to a sample from a patient with a diagnosis of calcified granuloma. The **arrows** indicate M_r protein standards in kilodaltons (kD). **B**, The strips (lanes) in **A** with their corresponding enzyme-linked immunosorbent assay optical density (O.D.) values and the number of proteins (bands) recognized by metacestode excretory/secretory (E/S) antigens. **C**, Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of metacestode E/S antigens. The gel was stained with silver. Lane 3, sample preincubated with E-64; lane 2, sample not incubated with E-64; lane 1, **arrows** indicate M_r protein standards in (kD).

with ELISA values ranging from 0.361 to 0.875 recognized three to nine metacestode E/S antigens (Figure 3A and B). In contrast, CSF samples with negative ELISA values (0.088–0.102) did not recognize E/S antigens (Figures 3A and B). Several of the antigens recognized had a molecular mass range of 6.7 to 130 kD. An antigen with an approximate molecular mass of 45 kD was recognized in six of seven CSF samples studied (Figure 3A). One CSF sample from a patient with a diagnosis of calcified granuloma and an ELISA value of 0.519 recognized four proteins in the EITB assay, but it could not be classified (Table 1 and Figure 3B).

Statistical results. The sensitivity of the ELISA was 92% (22 of 24) and the specificity was 97%. Cases with granuloma and/or calcifications (n = 8) were excluded since patients with calcifications do not actually have NCC but its sequel.[1]

DISCUSSION

Taenia solium metacestode E/S antigens have been evaluated for use in the diagnosis of human and porcine cysticer-

cosis.[12,33] However, they have not been studied in relation to the biologic stage of metacestodes in patients with NCC and specific IgG titers. In the current study, metacestode E/S antigens of *T. solium* used in ELISAs showed a strong capacity in discriminating between active and inactive forms of NCC.

Results showed that all cases with a single viable cyst had the highest OD values in the ELISA. The intermediate OD positive values corresponded to cases with viable cyst(s) plus transitional lesions. The lowest positive values corresponded to cases with viable cyst(s) plus one or more calcified cysts. In contrast, negative values were detected in all cases with calcified cysts.

There were two remarkable cases in this study. The first was a patient with a calcified granuloma, a highly positive ELISA value (0.519), and four proteins recognized in the EITB assay. In our study, positive ELISA values showed a correlation with the presence of a viable cyst. A calcified granuloma was reported in this case. However, the presence of viable cyst not detected by imaging studies cannot be ruled out. Moreover, similar discrepancies between the EITB assay and CT scans have been reported.[34] The second case had an ELISA value of 0.153, three viable cysts, and multiple calcifications identified by a CT scan. This patient probably had a low antibody response; unfortunately, the EITB assay could not be performed in this case.

It has been demonstrated that metacestodes of *T. solium* secrete a cysteine protease activity that depletes CD4+ cells *in vitro* and may be involved in parasite nutrition and immune evasion.[27] If the *T. solium* metacestode secretes proteins into its surroundings, the host may produce specific antibodies against these proteins. When the parasites are viable, they may be secreting proteases in addition to other proteins. Conversely, since degenerating or dead metacestodes do not secrete these proteins, the host response to this antigenic stimulation may be arrested. Only the remaining primed B cells may be producing specific antibodies against E/S antigens in a decreasing fashion. It is then likely that specific antibodies in patients with transitional or calcified NCC may be scarce or absent. Our results indicate that there is a direct relationship between the living stage of cysts and the presence of antibodies against E/S antigens.

The results of immunodiagnosis in inactive NCC have usually been reported as negative.[35] One study that used metacestode E/S antigens identified one positive case among 10 cases of inactive NCC.[12] However, in the current series, we did not find positive ELISA results in six cases with calcified lesions (inactive NCC). In contrast, all ELISA values were positive when a crude metacestode extract was used. This apparent discrepancy may be attributed to the prolonged release of somatic antigens in the process of parasite destruction. Moreover, it has been reported that specific antibodies persist long after the parasite has been eliminated by immune mechanisms and/or drug therapy.[36,37]

Although the number of CSF samples tested by immunoblotting was small, it is interesting to point out that a 45-kD antigen was recognized at a high frequency (86%). A good correlation was also found between high OD values and the number of antigens recognized by IgG antibody in the CSF samples.

Positive ELISA values were obtained for a CSF sample from a patient with meningitis due to *M. tuberculosis* and for another sample from a patient with bacterial meningitis plus positive serologic results for HIV. However, these were false-positive results that could be due to antigens from *M. tuberculosis* or other infective agents that cross-react with antibodies to metacestode E/S antigens.[38]

The ELISA and EITB assay with metacestode E/S antigens may be useful in diagnosing cases in which imaging studies cannot discriminate between active and inactive NCC,[20] and when making treatment decisions. The latter is particularly important since cysticidal drugs are effective only in patients with active forms of NCC. The immunodiagnostic assay may be also useful in endemic areas where equipment for imaging studies is not available. Finally, antibodies titers may be helpful in monitoring the biologic stage of the parasite in cases of extraparenchymal cysts in which the results of medical therapy are difficult to evaluate.[39,40]

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REFERENCES

1. Sotelo J, Guerrero V, Rubio F, 1985. Neurocysticercosis: A new classification based on active and inactive forms. *Arch Intern Med 145:* 442–445.
2. Salgado P, Rojas R, Sotelo J, 1997. Cysticercosis: Clinical classification based on imaging studies. *Arch Intern Med 157:* 1991–1997.
3. Carpio A, Escobar A, Hauser WA, 1998. Cysticercosis and epilepsy: a critical review. *Epilepsy 39:* 1025–1040.
4. Dixon HBF, Lipscomb FM, 1961. Cysticercosis: an analysis and follow-up of 450 cases. (*Medical Research Council Special Report Series, Volume 299*), London: Her Majesty's Stationary Service, 1–56.
5. Marquez-Monter H, 1971. Cysticercosis. Marcial-Rojas RA, ed. *Pathology of Protozoal and Helminthic Diseases.* Baltimore: Williams and Wilkins, 592–617.
6. Escobar A, 1983. The pathology of neurocysticercosis. Palacios E, Rodriguez-Carbajal, Taveras J, eds. *Cysticercosis of the Central Nervous System.* Springfield, IL: Charles C. Thomas, 27–54.
7. White AC, Jr., Tato P, Molinari JL, 1992. Host-parasite interactions in *Taenia solium* cysticercosis. *Infect Agents Dis 1:* 185–193.
8. Tsang VCW, Brand JA, Boyer AE, 1989. An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium). J Infect Dis 159:* 50–59.
9. Diaz JF, Verastegui M, Gilman RH, Tsang VCW, Piltcher JB, Gallo C, Garcia, HH, Torres, P, Montenegro T, Miranda E, and the cysticercosis working group in Peru, 1992. Immunodiagnosis of human cysticercosis (*Taenia solium*): a field comparison of an antibody-enzyme-linked immunosorbent assay (ELISA), an antigen-ELISA, and enzyme-linked immunoelectrotranfer blot (EITB) assay in Peru. *Am J Trop Med Hyg 46:* 610–615.
10. Simac C, Michel P, Andriantsimahavandy, Esterre P, Michault A, 1995. Use of enzyme–linked immunoelectrotranfer blot for the diagnosis and monitoring of neurocysticercosis. *Parasitol Res 81:* 132–136.

11. Ito A, Plancarte A, Ma L, Kong Y, Flisser A, Cho SY, Liu YH, Kamhawi S, Lightowlers MW, Schantz PM, 1998. Novel antigens for neurocysticercosis: simple method for preparation and evaluation for serodiagnosis. *Am J Trop Med Hyg 59:* 281–294.
12. Ko RC, Ng TF, 1998. Evaluation of excretory/secretory products of larval *Taenia solium* as diagnostic antigens for porcine and human cysticercosis. *J Helminthol 72:* 147–154.
13. Plancarte A, Fexas M, Flisser A, 1994. Reactivity ELISA and dot-plot of GP24, an immunodominant antigen of *Taenia solium*, for the diagnosis of human cysticercosis. *Int J Parasitol 24:* 733–738.
14. Ev LV, Maia AAM, Pianetti G, Nascimento E, 1999. Immunological evaluation of a 26-kDa antigen from *Taenia solium* larvae for specific immunodiagnosis of human neurocysticercosis. *Parasitol Res 85:* 98–102.
15. Larralde C, Sotelo J, Montoya RM, Palencia G, Padilla A, Govezensky T, Diaz ML, Sciutto E, 1990. Antigens from murine *Taenia crassiceps* cysticerci effectively substitute those from porcine *Taenia solium. Arch Pathol Lab Med 114:* 926–928.
16. Garcia E, Ordoñez G, Sotelo J, 1995. Antigens from *Taenia crassiceps* cysticerci used in complement fixation, enzyme-linked immunosorbent assay, and western blot (immunoblot) for diagnosis of neurocysticercosis. *J Clin Microbiol 33:* 3324–3325.
17. Gevorkian G, Manoutcharian K, Larralde C, Hernandez M, Almagro JC, Viveros M, Sotelo J, Garcia E, Sciutto E, 1996. Immunodominant synthetic peptides of *Taenia crassiceps in* murine and human cysticercosis. *Immunol Lett 49:* 185–189.
18. Manoutcharian K, Sotelo J, Garcia E, Cano A, Gevorkian G, 1999. Characterization of cerebrospinal fluid antibody specificities in neurocysticercosis using phage display peptide library. *Clin Immunol 91:* 117–121.
19. Hubert K, Andriantsimahavandy A, Michault A, Frosch M, Muhlschlegel FA, 1999. Serological diagnosis of human cysticercosis by use of recombinant antigens from *Taenia solium* cysticerci. *Clin Diag Lab Immunol 6:* 479–482.
20. Chung JY, Bahk YY, Huh S, Kang SY, Kong Y, Cho SY, 1999. A recombinant 10-kDa protein of *Taenia solium* metacestodes specific to active neurocysticercosis. *J Infect Dis 180:* 1307–1315.
21. Greene RM, Hancock K, Wilkins PP, Tsang VCW, 2000. Molecular cloning and serologic evaluation of 14-and 18-kDa related diagnostic antigens. *J Parasitol 86:* 1001–1007.
22. Sako Y, Nako M, Ikejima T, Piao XZ, Nakaya K, Ito A, 2000. Molecular characterization and diagnostic value of *Taenia solium* low–molecular-weight antigen genes. *J Clin Microbiol 38:* 4439–4444.
23. Restrepo BI, Llaguno P, Sandoval MA, Enciso JA, Teale JM, 1998. Analysis of immune lesions in neurocysticercosis patients: central nervous system response to helminth appears Th1-like instead of Th2. *J Neuroimmunol 89:* 64–72.
24. Grewal JS, Kaur S, Bhatti G, Sawhney IMS, Ganguly NK, Mahajan RC, Malla N, 2000. Cellular immune responses in human cysticercosis. *Parasitol Res 86:* 500–503.
25. Rodriguez V, de Mello FA, Magalhaes EP, Ribeiro SBF, Marquez JO, 2000. Interleukin-5 and interleukin-10 are major cytokines in cerebrospinal fluid from patients with active neurocysticercosis. *Braz J Med Biol Res 33:* 1059–1063.
26. White AC Jr, Molinari JL, Pillai AV, Rege AA, 1992. Detection and preliminary characterization of *Taenia solium* metacestode proteases. *J Parasitol 78:* 281–287.
27. Molinari JL, Mejia H, White AC Jr, Garrido E, Borgonio V, Baig S, Tato P, 2000. *Taenia solium*: A cysteine protease secreted by metacestodes depletes human CD4 lymphocytes in vitro. *Exp Parasitol 94:* 133–142.
28. Molinari JL, Soto R, Tato P, Rodriguez D, Retana A, Sepulveda J, Palet A, 1993. Immunization against porcine cysticercosis in an endemic area in Mexico: a field and laboratory study. *Am J Trop Med Hyg 49:* 502–512.
29. Molinari JL, Rodriguez D, Tato P, Soto R, Arechavaleta F, Solano S, 1997. Field trial for reducing porcine *Taenia solium* cysticercosis in Mexico by systematic vaccination of pigs. *Vet Parasitol 69:* 55–63.
30. Arechavaleta F, Molinari JL, Tato P, 1998. A *Taenia solium* metacestode factor nonspecifically inhibits cytokine production. *Parasitol Res 84:* 117–122.
31. Bradford M, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem 72:* 248–254.
32. Laemmli UK, 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature 227:* 680–685.
33. D'Souza PE, Hafeez M, 1999. Detection of *Taenia solium* cysticercosis in pigs by ELISA with an excretory-secretory antigen. *Vet Res Commun 23:* 293–298.
34. Garcia HH, Herrera G, Gilman RH, Tsang VCW, Pilcher JB, Diaz JF, Candy EJ, Miranda E, Naranjo J, The cysticercosis working group in Peru, 1994. Discrepancies between cerebral computed tomography and western blot in the diagnosis of neurocysticercosis. *Am J Trop Med Hyg 50:* 152–157.
35. Suss RA, Maravilla KR, Thompson J, 1986. MR imaging of neurocysticercosis: Comparison with CT and anatomopathological features. *AJNR Am J Neuroradiol 7:* 235–242.
36. Harrison LJS, Joshua GWP, Wright SH, Parkhouse RME, 1989. Specific detection of circulating surface/secreted glycoproteins of viable cysticerci in *Taenia saginata* cysticercosis. *Parasite Immunol 11:* 351–357.
37. Garcia HH, Gilman RH, Catacora M, Verastegui M, Gonzalez AE, Tsang VCW, The cysticercosis working group in Peru, 1997. Serological evolution of neurocysticercosis patients after antiparasitic therapy. *J Infect Dis 175:* 486–489.
38. Gottstein B, Tsang VCW, Schantz PM, 1986. Demonstration of species-specific and cross-reactive components of *Taenia solium* metacestodes antigens. *Am J Trop Med Hyg 35:* 308–313.
39. Teitelbaum GP, Otto RJ, Lin M, Watanabe AT, Stull MA, Manz HJ, Bradley WG Jr, 1989. MR imaging of neurocysticercosis. *AJNR A. J Neuradiol 10:* 709–718.
40. Monteiro L, Almeida-Pinto J, Stocker A, Sampaio-Silva M, 1993. Active neurocysticercosis, parenchymal and extraparenchymal: a study of 38 patients. *J Neurol 241:* 15–21.