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Characterization by Enzyme-Linked Immunosorbent Assay of the Humoral Immune Response in Patients with Neurocysticercosis and Its Application in Immunodiagnosis

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The enzyme-linked immunosorbent assay was standardized for the search for specific antibodies in human neurocysticercosis. A crude cysticercal extract and two partially purified antigenic fractions were used, as well as serum and cerebrospinal fluid (CSF) samples of different groups of subjects. Immunoglobulin G (IgG) antibodies were detected in serum and CSF, with a sensitivity of 85 and 90%, respectively. Specificity was 96% with a partially purified antigen and 100% with the crude cysticercal extract. IgM and IgA antibodies were detected less frequently, and IgE was detected only occasionally, both in serum and CSF. Analysis of serum and CSF samples drawn from the same patient did not always reveal the presence of anticysticercus antibodies in both samples. A significant correlation was found between the presence or absence of IgG antibodies in the CSF and the morphological appearance of the parasite (undamaged or calcified). Variations in the humoral response were not found to correlate with clinical and laboratory findings.

The detection of specific antibodies in patients with neurocysticercosis is a useful tool for diagnosis of the disease, especially when computed tomography is not available or conclusive. Several techniques have been used for the immunologic diagnosis of neurocysticercosis (11, 12), such as complement fixation (23), immunofluorescence (15, 24), and the enzyme-linked immunosorbent assay (ELISA), which was recently standardized (3, 6, 8, 21).

The detection of specific antibodies in patients with neurocysticercosis allows the characterization of their immune response toward cysticerci. Specific antibodies of all immunoglobulin classes have been found in the serum of patients with neurocysticercosis by modified immunoelectrophoresis (13). Sera may react with up to eight cysticercal antigens of different electrophoretic mobilities, antigen B (AgB) being the most frequent (13). This antigen has been purified (16) and used in an ELISA (8) in which the presence of immunoglobulin G (IgG) antibodies against AgB was demonstrated in 8 of 11 serum samples and 11 of 13 cerebrospinal fluid (CSF) samples drawn from patients with neurocysticercosis.

Very few seroepidemiologic surveys for cysticercosis have been reported (4, 10, 14, 28). Countries with a high incidence of the disease would profit from such studies through the identification of groups with a high risk of infection and the detection of factors that influence the transmission of the disease. However, immunologic methods used in seroepidemiology should be sensitive and specific; difficulty in attaining these parameters may be the reason for the scarcity of such studies.

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This study was designed to evaluate the sensitivity and specificity of the ELISA for neurocysticercosis immunodiagnosis and seroepidemiology, by using a crude extract (CE) and two partially purified fractions of *Taenia solium* cysticerci, as well as serum and CSF drawn from patients with other parasitic diseases.

In addition, we defined and compared the classes of anticysticercus antibodies present in the serum and CSF of patients with neurocysticercosis and analyzed the correlation between the humoral immune response and the main clinical and laboratory findings exhibited by these patients.

MATERIALS AND METHODS

Study population and clinical samples. A total of 162 serum samples were obtained from the following groups of individuals: 61 patients with neurocysticercosis (confirmed at surgery or with clinical and laboratory data suggestive of the disease, including the detection of anticysticercus antibodies in serum by immunoelectrophoresis); 76 healthy individuals; and 25 patients with other parasitic diseases (5 with hydatid disease, 5 with strongyloidiasis, 5 with trichinosis, 5 with filariasis, and 5 with visceral larva migrans). The diagnosis of patients with hydatid disease was confirmed at surgery. The diagnosis of patients with other helminthic diseases was determined from information provided by the attending physicians and positive results of serodiagnostic testing performed by the Helminthic Diseases Branch, Centers for Disease Control, Atlanta, Ga. The diagnosis of filariasis was based on clinical data and history of exposure in an area to *Wuchereria* or *Brugia* spp.

CSF samples were obtained from 82 patients with neurocysticercosis confirmed by surgery or by the presence

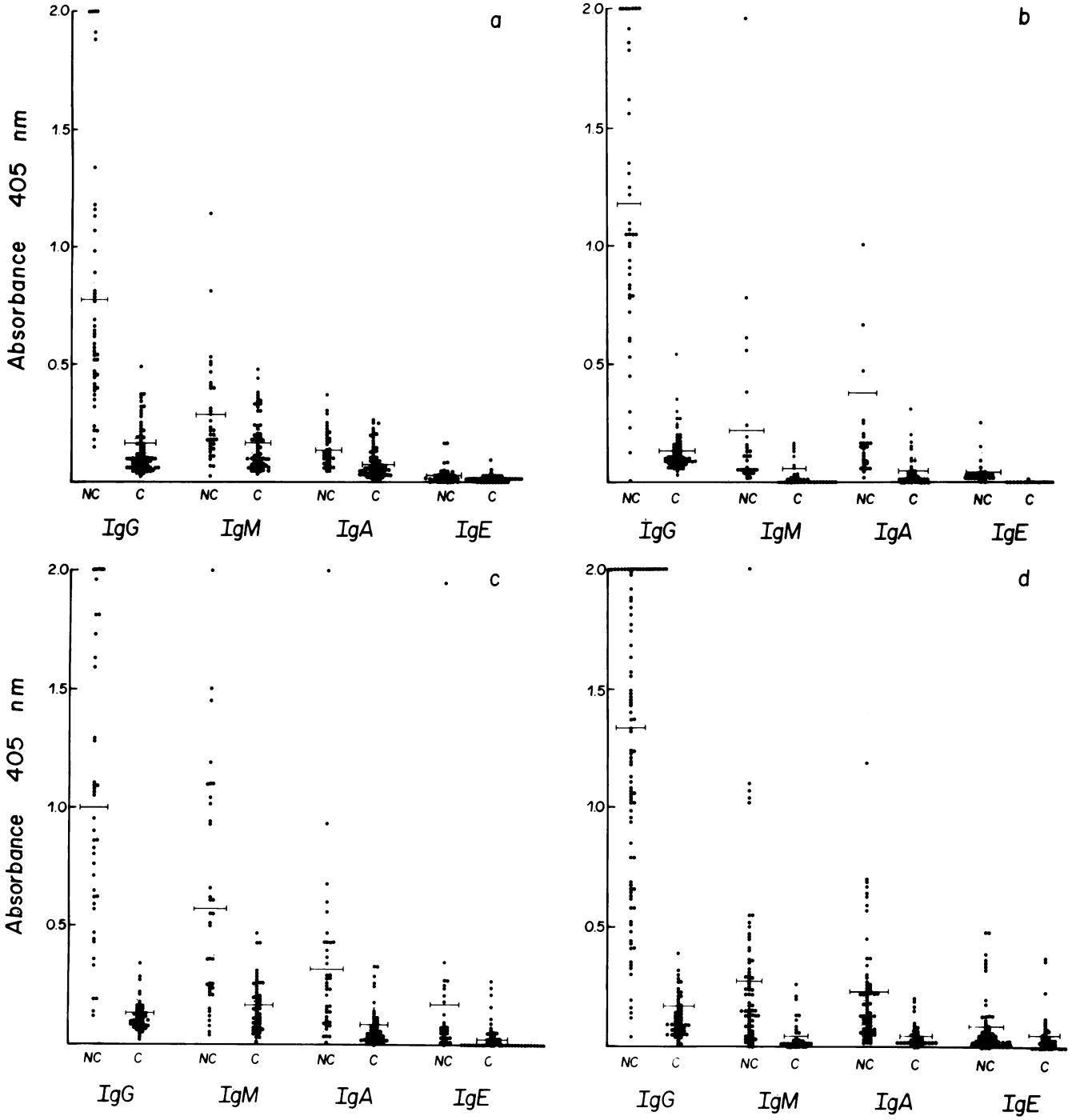

FIG. 1. A_{405} values obtained in ELISA of serum (a, c) and CSF (b, d) samples from patients with neurocysticercosis (NC) and control groups (C) with AgB (a, b) or a cysticercal CE (c, d). Conjugates of alkaline phosphatase and anti-human IgG, IgM, IgA, or IgE were used.

of specific antibodies in their serum; 70 samples were drawn from healthy individuals undergoing subdural anesthesia; and 16 were drawn from patients with other neurologic disorders.

Serum and CSF samples were obtained simultaneously from each of 38 patients with confirmed neurocysticercosis.

Antigens. A CE obtained from *T. solium* as previously described (8, 13) was used. A partially purified fraction of AgB (95 and 85 kilodaltons and three minor bands) (16) and cysticercus surface glycoproteins (kindly provided by J. P. Laclette, Instituto de Investigaciones Biomédicas, UNAM) were also used.

ELISA. The solid-phase ELISA (27) was performed as described elsewhere (8). A 1-μg sample of protein (CE, AgB, or glycoprotein) per ml was adsorbed to Immulon plates (Dynatech Laboratories, Inc., Alexandria, Va.). Alkaline phosphatase conjugates (Sigma Chemical Co., St. Louis,

TABLE 1. Cross-reactions of sera from patients with different parasitic diseases and antigens obtained from *T. solium* cysticerci as measured by ELISA[a]

Parasitic disease or organism	No. of samples positive to:		
	CE	AgB	Glycoproteins
Hydatidosis	4	3	2
Strongyloidosis	5	0	0
Trichinosis	4	0	0
Filariasis	1	0	0
T. canis	0	0	0

[a] Five serum samples were tested.

Mo.) were diluted 1:1,000 for use. Their specificity was confirmed by adsorbing IgG, IgM, and IgA (Sigma) and the serum from three allergic patients with high IgE titers to the plates and subsequently adding the conjugates. Serum was diluted 1:2,000 and CSF was diluted 1:10, as described in a previous report (8).

Clinical status of the patients. Clinical histories were obtained, as well as data on cell counts and protein and glucose concentrations in CSF, neuroradiologic data, and treatment results, for 26 patients with neurocysticercosis. Patient ages ranged between 10 and 60 years; 65% were females and 35% were males. Intracranial hypertension was observed in 23 patients (88%), seizures were present in 5 patients (19%), and 2 patients had both syndromes. In association with intracranial hypertension, five patients had decreased vision, three had loss of consciousness, two had cerebellar alterations, two had psychiatric disorders, two had neurologic deterioration, two had dizziness, two had muscle weakness, two had myalgias, and one had nystagmus. Symptoms had appeared between 2 and 20 years (average, 2 years) before the study. Cell counts in the CSF were normal for 8 (30%) of the patients; they had increased to 5 to 50 cells per ml for 11 (42%) patients, to 51 to 200 cells per ml for 5 (19%) patients, and to more than 200 cells per ml for only 2 patients. Eosinophils were increased in four patients. The protein concentration in the CSF was within normal limits for 16 patients (64%) and slightly increased (45 to 75 mg/dl) for 6 patients (24%); for 2 patients it was 75 to 100 mg/dl, and for 6 patients it was greater than 500 mg/dl. The glucose concentration in the CSF was normal for 19 patients (79%), slightly diminished (40 to 45 mg/dl) for 3 patients (12%), and less than 40 mg/dl for 2 patients (8%). Normal and pathologic values were taken from Fishman (9).

Ventricular cysticerci were observed in 12 (48%) of the patients by computed tomography; none of the cysticerci were calcified. In nine patients (36%), the cysticerci were located within the parenchyma, and six of these were calcified. Four patients harbored cysticerci in both locations. All patients were treated with steroids; six patients (23%) also underwent anticonvulsive treatment; 61.5% of the patients had a shunt; and in 15.5% of the patients, the parasite was excised before the samples were obtained.

TABLE 3. Frequency of anticysticercus antibodies in CSF and serum of the same patient

Pattern[a]	% of positive samples in immunoglobulin class			
	IgG (n = 38)	IgM (n = 37)	IgA (n = 38)	IgE (n = 23)
CSF+ Serum+	58	22	10	0
CSF+ Serum−	21	5	10	4
CSF− Serum+	13	24	13	4
CSF− Serum−	8	49	66	91

[a] +, Presence of the immunoglobulin class; −, absence of the immunoglobulin class.

RESULTS

ELISA sensitivity and specificity. Mean absorbance values for anti-AgB or anti-CE IgG antibodies were significantly different in the CSF and serum of patients with neurocysticercosis compared with controls. Absorbance values for IgM, IgA, and IgE antibodies were higher in some patients than in controls, but mean values were not significantly different (Fig. 1). A result was considered positive when the A_{405} was >0.4. Sensitivity (number of serologically positive patients/total number of patients with proved disease) and specificity (number of serologically negative individuals/total number of healthy individuals) were evaluated with data obtained for IgG in the serum and CSF of patients with neurocysticercosis, other neurologic patients, and healthy subjects. The sensitivity of the serum ELISA was 85% with CE and 80% with AgB, and the sensitivity of the CSF ELISA was 90% with either antigen. Specificity was 100% with CE in both samples and 96 and 98% with AgB in serum and CSF, respectively.

To assess whether the ELISA may be useful for epidemiologic studies, it was applied to serum samples from individuals with other parasitic diseases. Twenty-five serum samples were tested with the CE, with AgB, and with an enriched surface glycoprotein fraction obtained from *T. solium* cysticerci; the conjugate was anti-IgG. The results (Table 1) indicate that with the CE, cross-reactions were frequent but were eliminated with the use of partially purified fractions, except in some cases of hydatid disease.

Characterization of immune response by ELISA. The frequency of detection of anticysticercus antibodies of each immunoglobulin class in patients with neurocysticercosis is shown in Table 2. IgG antibodies against the CE or AgB were present in most analyzed serum and CSF samples. IgM and IgA antibodies were detected less frequently in both serum and CSF. IgE antibodies were detected only occasionally.

Anticysticercus antibodies of different immunoglobulin classes were measured in serum and CSF samples drawn simultaneously from 38 patients (Table 3). IgG antibodies were detected in both samples from 22 patients (58%) and in either serum or CSF from 13 patients (34%) and were absent from both for only 3 patients (8%). IgM, IgA, and IgE

TABLE 2. Detection of systemic and local anticysticercus antibodies by ELISA for patients with neurocysticercosis

Antigen	No. of positive samples/total no. tested (%)							
	IgG		IgM		IgA		IgE	
	Serum	CSF	Serum	CSF	Serum	CSF	Serum	CSF
CE	35/41 (85)	82/91 (90)	19/38 (50)	14/70 (20)	10/39 (26)	9/69 (13)	1/31 (3)	2/60 (3)
AgB	36/45 (80)	37/41 (90)	10/35 (29)	4/30 (13)	0/37	3/29 (10)	0/36	0/27

TABLE 4. Correlation of various parameters of CSF of patients with neurocysticercosis in relation to pathologic characteristics of the cysticercus and the main symptoms induced

Characteristic	No. of patients	% Patients with:								
		Anticysticercus antibodies						Cells >5[a]	Protein >45 mg/dl[a]	Glucose <45 mg/dl[a]
		IgG		IgM		IgA				
		CSF	Serum	CSF	Serum	CSF	Serum			
Morphologic appearance										
Calcified	9	44[b]	67	44	33	55	33	67	44	0
Undamaged	17	94[b]	59	29	29	23	41	70	29	23
Localization										
Parenchymatous	9	67	55	33	22	33	22	67	44	0
Ventricular	12	92	67	33	33	17	50	67	27	25
Mixed	4	50	75	50	50	50	75	100	25	50
Symptoms										
Endocraneal hypertension	23	78	65	26	30	26	39	78	30	22
Seizures	5	60	40	20	40	40	20	60	40	11

[a] For normal and pathologic values, see reference 9.
[b] $P < 0.01$.

antibodies were detected less frequently than IgG antibody and were absent from both serum and CSF for 18 patients (49%), 25 patients (66%), and 21 patients (91%), respectively. Antibodies of the IgM class were rarely found only in the CSF, whereas IgA antibodies appeared with similar frequencies with both samples or just one. IgE was detected in one serum and one CSF sample, not from the same patient.

Immune response and clinical status of the patient. The clinical status of the patients varied considerably. Symptoms and location of the parasites were not found to correlate with the presence of any particular class of anticysticercus antibodies. The presence of IgG antibodies in the CSF showed a significant correlation to the morphologic appearance of the cysticerci: 94% of the patients harboring morphologically undamaged cysticerci had IgG antibodies, in contrast to 44% of the patients harboring calcified parasites (Table 4).

DISCUSSION

Anticysticercus antibodies of several classes of immunoglobulins were investigated in CSF and serum to characterize the humoral immune response in patients with neurocysticercosis.

TABLE 5. Anticysticercus antibodies in samples obtained simultaneously from 38 patients with neurocysticercosis

Sample(s) and immunoglobulin classes detected	% Positive
Serum	
IgG	71
IgG, IgM	74
IgG, IgA	74
IgG, IgM, IgA	76
CSF	
IgG	79
IgG, IgM	84
IgG, IgA	87
IgG, IgM, IgA	92
Serum and CSF	
IgG	92
IgG, IgM	97
IgG, IgA	100
IgG, IgM, IgA	100

The performance of the ELISA described here was superior to that of other previously standardized serologic techniques for cysticercosis (12), and the sensitivity and specificity compared favorably with results reported from other laboratories (reviewed in reference 11). IgG class antibody reactivity was detected more frequently in the CSF (90%) than in the serum (85%) samples. Combining the results of IgG and IgA tests with serum and CSF samples, antibody reactivity was detected for 100% of 38 patients from whom paired specimens, drawn simultaneously, were available (Table 5).

The specificity of the ELISA was very high, as shown by the absorbance values obtained with samples originating from symptomatic patients with neurocysticercosis as opposed to samples from patients with nonparasitic neurologic disorders and from healthy subjects.

Considering that the ELISA could be used for seroepidemiologic surveys, it was judged important to evaluate sera from patients with other parasitic diseases. High specificity was observed with AgB but not with the CE. The CE may serve as a screen for all sera, and those positive should be further tested with AgB. Positive sera indicate either cysticercosis or hydatidosis. There are no reports on hydatid disease in Mexico. Cross-reactions among these parasites may be explained by their phylogenetic proximity (18) and were previously reported (24, 25). Our data stress the need for further identification and purification of specific antigens to be used in seroepidemiologic studies. For immunodiagnostic purposes, the presence of anticysticercus antibodies in patients with neurologic symptomatology suggestive of cysticercosis strengthens the diagnosis of the disease. Consequently, for the diagnosis of clinical cases, the ELISA may be performed with the CE.

Certain pathologic conditions of the central nervous system are associated with increased levels of immunoglobulins in the CSF (1, 9, 17, 22), some of which are locally produced (1, 17, 20). Data from the present study (Table 3, CSF+ serum−) also suggest local production of specific immunoglobulins, mainly IgG and IgA. Miller et al. (20) provided data on intra-blood-brain-barrier IgG antibody synthesis in

five of six patients with neurocysticercosis; the antigenic specificity of this IgG was not determined. The results shown in Table 3 refer to anticysticercus antibodies. They suggest that the lymphoid tissue of the brain reacts with cysticercal antigens.

High IgE values, including those for specific IgE antibodies in cysticercosis (2, 13), have been reported in many helminthic diseases (5). In this study, no specific IgE was found, although controls for ELISA-allergic patients with high titers of IgE had an A_{405} of >1 when tested with anti-IgE. This may have been due to the localization of cysticerci only in the CNS where no IgE is produced and, for the same reason, systemic IgE is not induced. Alternatively, IgE may have been present in the samples but not detected because of the high dilution used (1:2,000 for serum, 1:10 for CSF) or because of competition with IgG for antigenic determinants, the latter being more abundant or having a higher affinity and impeding the reaction of IgE with the antigens.

The clinical heterogeneity of neurocysticercosis has been extensively documented (26, 29). The single clinical correlation ($P < 0.01$) with antibody level found in the group of patients in the present study was the frequent absence of IgG antibodies in the CSF of patients with calcified cysts (Table 4). The probable explanation is that calcified parasites are not antigenic. In the patients with a persistent immune response, this may have been due to the presence of other viable but asymptomatic or otherwise undetected cysticerci. In contrast, patients with viable cysts generally had detectable anticysticercus IgG, indicating that living parasites stimulated the immune response of the host. Similar results were reported when antibodies in the CSF were measured by radioimmunoassay (19): 53% of the patients with ventricular cysts were positive, whereas only 10% of those harboring parenchymatous calcified cysts gave a positive result.

Furthermore, the presence of IgG indicates that the immune response is secondary; this is supported by the fact that cysticercosis is a long-lasting disease (7).

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