Immunodiagnosis of Human Cysticercosis (Taenia solium) with Antigens Purified by Monoclonal Antibodies

EVALDO NASCIMENTO,1 CARLOS A. TAVARES,1 AND JOSÉ D. LOPES2*

Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG,1 and Ludwig Institute for Cancer Research, São Paulo Branch, 01509, São Paulo,2 Brazil

Received 11 September 1986/Accepted 17 March 1987

Monoclonal antibodies were generated from mice immunized with scolex protein antigen of Cysticercus cellulosae. Three monoclonal antibodies specific for cysticercal antigens, which did not show any cross-reactivity with Taenia solium or Taenia saginata antigens, were selected. Each monoclonal antibody coupled to Sepharose could purify one antigen, which appeared as a single band on polyacrylamide gel electrophoresis. When antigens purified by monoclonal antibodies were used to detect antibody in serum samples taken from patients with cysticercosis, taeniasis, and other parasitic infections in an enzyme-linked immunosorbent assay, cross-reactivity was observed until a serum dilution of 1:128 was reached. Since serum samples from unexposed subjects showed positive reactions until a dilution of 1:64 was reached, we chose a discriminative dilution (1:128) above which no cross-reaction was observed. The percent positive serum samples from cysticercosis patients was 100% by the enzyme-linked immunosorbent assay with any of the antigens purified by monoclonal antibodies.

Human and pig cysticercosis and human infection with Taenia solium constitute important public health problems in large parts of Asia, Africa, and Latin America, where environmental sanitation is poor (18). Neurocysticercosis is the more severe form of the disease, occurring with high frequency in Mexico, Brazil, and Chile. Neurological disturbances are the main clinical features, and brain lesions can be found at autopsy (3).

As cysticercosis is a common disease in several countries and produces heterogeneous clinical manifestations, the development of a specific and sensitive method for the identification of this disease is desirable. Throughout this century, immunological methods have been used for the diagnosis of cysticercosis (2, 9–12; E. Nascimento, P. M. P. Nogueira, and C. A. P. Tavares, Z. Parasitenkde., in press). However, all methods so far tested lack sensitivity or specificity or both (12). False-positive reactions, mainly with serum samples from patients infected with T. solium and T. saginata, are observed, and samples from some patients show false-negative results (16; Nascimento et al., in press). Several reasons for false-negative results have been postulated, including the inherent lack of sensitivity of the methods used (9), the existence of immunologically nonresponsive patients (10), and the use of nonrepresentative antigen preparations (20). The use of a crude extract as a source of antigen may explain the lack of sensitivity or specificity, or both, of the immunodiagnostic methods (19).

Better results were obtained by several authors with the enzyme-linked immunosorbent assay (ELISA). Arambulo et al. (1) detected antibodies against cysticercus by using ELISA with an aqueous extract of cysticerci as the antigen preparation, and Diwan et al. (6) used a saline extract of cysticerci to detect antibodies in serum samples from patients with established diagnosis of neurocysticercosis. Recently one antigen, named antigen B, purified from cysticerci of T. solium by Guerra et al. (12) was used to detect antibodies against cysticerci in serum and cerebrospinal fluid samples from patients with cysticercosis (7).

The purification of parasite antigens for serological determinations could improve specific immunodiagnosis of cysticercosis. Monoclonal antibodies (MAbs) have great potential in the development of specific immunodiagnostic reagents. Indeed, a prototype MAb-based immunodiagnostic test with high sensitivity and specificity has been described for a model system involving a natural larval cestode parasite of mice (14). The potential of a similar system has also been demonstrated in the immunodiagnosis of the infection of sheep with T. hydatigena (5). Antigens isolated by use of mAbs were used in the immunodiagnosis of Echinococcus granulosus infection (hidatid) in sheep (4).

In the present study, MAbs reactive against a scolex protein antigen (SPA) of the cysticerci of T. solium were produced and conjugated to activated Sepharose to prepare an affinity column. The crude SPA was applied to the column, and three different antigens were obtained. A significant increase in specificity was obtained when these antigens were used in an ELISA for the detection of antibodies in sera of patients with cysticercosis.

MATERIALS AND METHODS

Antigens. The cysticerci were excised from skeletal muscles and organs of naturally infected pigs. The SPA was obtained by dissection of cysticerci with scalpels under a stereoscopic microscope. The scolices were washed several times in distilled water, lyophilized, and ground to powder. A 2-g portion of the powder was suspended in 30 ml of 0.15 M phosphate-buffered saline (PBS) (pH 7.2) and given three 1-min sonication bursts. After centrifugation at 4,800 × g for 20 min at 4°C, the supernatant was removed, dialyzed against PBS, and stored at −20°C until used.

Cysticercus membrane antigen (CMA), vesicle liquid antigen (VLA), T. solium antigen (TSOA), and T. saginata antigen (TSAA) were obtained as previously described (15).

Production of MAbs. BALB/c female mice were each injected subcutaneously with 100 µg of SPA in Freund incomplete adjuvant. Four injections were given at 2-week intervals. At 4 days before fusion, each mouse was intrave-
nously boosted with 50 µg of SPA without adjuvant. Spleen cells from immunized mice were fused with the SP2/0 myeloma cell line with polyethylene glycol 4000 (Sigma Chemical Co.) as previously described (8). Hybridomas secreting antibodies positive in ELISA with SPA antigen were cloned by limiting dilution with BALB/c peritoneal macrophages as feeder layers. Cloned hybridomas were cultured to obtain large volumes of culture supernatants. Large quantities of MAbs were produced by growing hybridomas in the peritoneal cavity of BALB/c mice. The mice were each injected intraperitoneally with 0.5 ml of pristane and 7 days later with 10³ hybridoma cells, and ascitic fluid was collected 2 to 3 weeks later.

**Isotype analysis.** Immunoglobulin isotypes were determined from concentrated tissue culture supernatants of each MAb by Ouchterlony gel diffusion in 1% agarose in PBS. Rabbit anti-mouse immunoglobulin M (IgM), IgG1, IgG2a, IgG2b, and IgG3 (Institut Pasteur, Paris) were used.

**ELISA.** ELISA was used (i) to screen hybridomas producing MAbs, (ii) to detect MAb cross-reactivity to different cysticerci antigens such as membrane, scolex, and vesical liquid as well as *T. solium* and *T. saginata* antigens, and (iii) to test the reactivity of serum samples from patients with cysticercosis and other parasitic infections to antibodies purified by MAbs. The protein concentration of the antigens for the ELISA assays was previously determined by titration by using a pool of human sera positive for cysticercosis.

Antigens purified by MAbs were used at concentrations of 1 µg of protein per well, and all other antigens were used at 3 µg of protein per well. ELISA was carried out in polyvinyl chloride microtitration plates (Costar). Microtiter wells were coated with antigen in carbonate buffer (pH 9.6) and incubated overnight at 4°C. The wells were then washed three times with PBS containing 1% casein (Merck & Co., Inc.) and 0.05% Tween 20 (PBS-T). A 100-µl portion of tissue culture supernatants, ascitic fluids, or serum samples from patients with either cysticercosis or other parasitic infections were added to the wells, and the plates were incubated at room temperature (25°C) for 1 h. The wells were again washed three times with PBS-T, 100 µl of 1:600 rabbit anti-mouse or anti-human immunoglobulins conjugated with horseradish peroxidase (Sigma type VI) in PBS-T was added to each well, and the plates were incubated for 30 min at room temperature. After the wells had been washed six times in PBS-T, 150 µl of 0.002% O-phenylenediamine (Sigma) in buffer solution (pH 5.0) containing 13 mM Na₂HPO₄, 24 mM citric acid, and 0.012% N₂O₂ was added to each well. The reaction was interrupted after 20 min at room temperature by the addition of 20 µl of 4 N H₂SO₄. The A₄₉₀ was measured in a Uniskan photometer (ELFAB).

**Immunofluorescence chromatography.** Supernatants containing MAbs were concentrated by ammonium sulfate precipitation, dialyzed against PBS, and conjugated to CH-Sepharose 6B (Pharmacia, Uppsala, Sweden) as specified by the manufacturer at the rate of 10 mg of antibody to 1 g of gel. SPA (6 mg in 2 ml of PBS) was affinity purified by incubation in the coupled gel overnight at 4°C. Bound antigen was eluted with 0.2 M glycine (pH 9.0), after several other solutions with different pH and molarities were tested. Elution profiles were monitored with an LKB Uvicord III. Eluates were neutralized by 0.05 N HCl, dialysed exhaustively against 0.05 M ammonium bicarbonate, and lyophilized.

**Sodium dodecyl sulfate-polyacrylamide gel electrophoresis.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed, as described by Laemmli (13), in a 7 to 15% gradient for separating gels. Molecular weight markers (Sigma) were always used. After electrophoresis, gels were stained with Coomassie blue.

**Human sera.** Serum samples were obtained from 22 patients with neurocysticercosis, presenting live cysticerci as confirmed by surgery. In addition, serum samples from 15 patients with syphilis, 12 with schistosomiasis, 10 with ascariasis, 4 with ancylostomiasis, 6 with strongyloidiasis, 12 with Chagas' disease, 33 with taeniasis due to *T. solium*, and 15 with taeniasis due to *T. saginata* (from the Institute of Biological Science, Belo Horizonte, MG, Brazil) were tested. Twenty-four serum samples obtained from newborn children and provided by F. Araujo, Research Institute, Palo Alto Medical Foundation, Palo Alto, Calif., were used as negative controls. All serum samples were stored at −20°C until use.

**RESULTS**

**Characterization of MAbs.** Hybridoma culture supernatants were screened by ELISA. Twenty-seven MAbs recognized antigens present in SPA of the cysticerci, as well as in *T. solium* TSOA or *T. saginata* TSAA. Other antigens such as CMA and VLA were tested. Three MAbs with IgM isotype (2D5C2, 2D5D9, and 1F5C3) were selected which recognized epitopes present in SPA, CMA, and VLA but not in TSOA or TSAA. 1F5C3 did not recognize VLA (Table 1).

**Purification of antigens.** Antigens in the SPA preparation were purified by affinity chromatography. MAb 2D5C2 isolated a protein with an approximate molecular mass of 80 kilodaltons (kDa) (SPA 80), and MAbs 2D5D9 and 1F5C3 isolated proteins with similar molecular mass (10 kDa) (SPA 10a, SPA 10b) (Fig. 1). However, although MAbs 2D5D9 and 1F5C3 recognized antigens with similar migration patterns in sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the results obtained in the ELISA suggest that the epitopes recognized by 1F5C3 and 2D5D9 are different because 2D5D9 did react with VLA, whereas 1F5C3 did not.

**Sensitivity of the isolated antigens.** Affinity-purified antigens were used in an ELISA to determine whether their use would increase the sensitivity of the test for detection of antibodies to *Cysticercus cellulosae* (Table 2). An increase in titer was obtained with the MAbs when affinity-purified antigens were used. However, statistically significant differences between the titers obtained with SPA and antigens purified by MAbs were seen only with MAbs from hybridoma supernatants (Table 2).

**Detection of antibodies in human sera.** Affinity-purified antigens were used in an ELISA to detect antibodies in serum samples from patients with cysticercosis, taeniasis, and other parasitic diseases (Fig. 2). Antigens purified by MAbs, as well as SPA, can detect specific antibodies present in serum samples from patients with cysticercosis. A discriminative dilution above which no cross-reactivity was observed with serum samples from patients with taeniasis,

<table>
<thead>
<tr>
<th>Antigen</th>
<th>2D5C2</th>
<th>2D5D9</th>
<th>1F5C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPA</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CMA</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>VLA</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TSOA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TSAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
from patients with other parasitoses, and from control individuals was chosen. In the assay with affinity-purified antigens, MAb titers of 1:512 or higher were common. The titers with the SPA antigen were lower, and two samples from patients with cysticercosis gave titers of 1:256 (Fig. 2). Thus considering 1:128 to be the discriminative dilution, the percent positivity by ELISA for 22 serum samples from patients with cysticercosis was 100% when antigens affinity purified with MAb were used and 91% when crude SPA was used (Table 3).

**DISCUSSION**

The immunodiagnosis of cysticercosis has been done by a variety of methods including complement fixation (Nascimento et al., in press), indirect hemagglutination (2, 16, 17; Nascimento et al., in press), immunoelectrophoresis (10), and ELISA (1, 6, 7; Nascimento et al., in press). Each of these methods has its peculiar combination of sensitivity and specificity, but the greatest difficulty resides in the serological differentiation of patients with taeniasis as well as other helminthic infections such as schistosomiasis or ancylostomiasis.

In an attempt to avoid this cross-reactivity different antigenic preparations have been devised (1, 6, 12, 15; Nascimento et al., in press). Biochemical methods were used to obtain purified antigen from complex antigenic preparations of *C. cellulosae* (12, 15).

MAbs were prepared with SPA from *T. solium* larvae, because this antigen preparation has been characterized as an inducer of high antibody titers in rabbits and because it has been shown to be the best antigen in assays detecting antibodies in serum samples of patients with cysticercosis (15, 16).

In the work described in this paper, three antigens were isolated by affinity chromatography: one with a molecular mass of 80 kDa and two others with a molecular mass of 10 kDa. The two 10-kDa antigens are probably different, because the respective MAbs recognize different epitopes on CMA, as the ELISA results revealed: MAb 2DS9 binds to VLA, and MAb 1F5C3 does not (Table 1). Antigens were eluted at high pH, which did not affect their reactivity in ELISAs.

Use of purified antigens led to an increased titer of antibodies as detected by ELISA in hybridoma culture supernatants and mice ascites, suggesting that their use in serological tests could increase the sensitivity for antibody detection. However, when purified antigens were used to detect antibody in serum samples from patients with cysticercosis and other parasitic infections, cross-reactivity below a 1:128 dilution was observed. Even serum samples from unexposed subjects showed positive reactions at 1:64 dilutions. Because of this, a discriminative dilution of 1:128 was chosen, above which no cross-reactions were observed (Fig. 2). When this discriminative dilution was used, it was possible to differentiate patients with cysticercosis from patients with other parasitic diseases. With antigens purified by affinity chromatography with MAbs titers ranged from 1:512 to 1:131,672, and the lowest positive dilution was twice the discriminative dilution. With SPA, two serum samples presented titers equal to that of the discriminative dilution (1:128).

The percent positivity for 22 serum samples from patients with cysticercosis as measured by ELISA with SPA 80, SPA 10a, or SPA 10b was 100%, but with crude SPA it was only 91%. Using the same assay but with crude antigen of cysticerci, Arambulo et al. (1) detected antibodies in 77.5% of serum samples from patients suspected of having cysticercosis, and Diwan et al. (6) detected antibodies in 79% of serum samples from patients with an established diagnosis of neurocysticercosis. A purified antigen from cysticerci of *T. solium* antigen B detected antibodies in 73% of the serum samples and in 85% of the cerebrospinal fluid samples tested (12). In the present study, a partially purified antigen (SPA) showed a percent positivity of 91%, being the best antigen obtained by conventional methodology for the immunodiagnosis of cysticercosis. It is important to note that SPA is obtained by a very simple methodology accessible to any laboratory.

With antigens purified by MAbs, no false-negative or false-positive reactions were observed. These results emphasize the importance of producing parasite species-specific MAbs applicable for direct immunodiagnostic use. Too much is left to luck if one believes that a mouse will respond to appropriate determinants. Moreover, the greater restriction of antigenic determinants being used in immunodiagnosis, the greater is the probability of obtaining false-negatives in the test. Therefore, Craig et al. (5) suggested that a more fruitful approach may be to select from murine hybridomas producing cross-reactive antibodies by

**TABLE 2. ELISA titers with SPA and with antigens isolated from SPA by affinity chromatography with MAbs**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>MAb</th>
<th>Hybridoma supernatants&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ascites</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPA</td>
<td>2DSC2</td>
<td>32</td>
<td>131,072</td>
</tr>
<tr>
<td>SPA</td>
<td>2DS9</td>
<td>64</td>
<td>262,144</td>
</tr>
<tr>
<td>SPA</td>
<td>1F5C3</td>
<td>256</td>
<td>524,288</td>
</tr>
<tr>
<td>SPA 80</td>
<td>2DSC2</td>
<td>1,024</td>
<td>1,048,576</td>
</tr>
<tr>
<td>SPA 10a</td>
<td>2DS9</td>
<td>512</td>
<td>262,144</td>
</tr>
<tr>
<td>SPA 10b</td>
<td>1F5C3</td>
<td>2,048</td>
<td>1,048,576</td>
</tr>
</tbody>
</table>

<sup>a</sup> Titer differences were significant (*P* < 0.05) for hybridoma supernatants.
using a range of heterologous antigen preparations and clinically defined serum samples in direct and competitive binding assays. These antibodies could then be used to process crude homologous parasite antigens for direct immunodiagnostic use. In the present study, antigens purified by affinity chromatography with MAbs were successfully used in an immunodiagnostic test which could distinguish serum samples from patients with cysticercosis from serum samples from patients with other parasitoses, including *T. solium* or *T. saginata* taeniasis. Thus, the antigens purified by the described MAbs may prove to be useful in medical practice as a confirmatory test for patients for whom cysticercosis is suspected.

**TABLE 3.** Percent positivity in 22 serum samples from patients with proven cysticercosis

<table>
<thead>
<tr>
<th>Antigen</th>
<th>No. (%) of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPA</td>
<td>20 (91)</td>
</tr>
<tr>
<td>SPA 80</td>
<td>22 (100)</td>
</tr>
<tr>
<td>SPA 10a</td>
<td>22 (100)</td>
</tr>
<tr>
<td>SPA 10b</td>
<td>22 (100)</td>
</tr>
</tbody>
</table>

FIG. 2. Titters of antibodies detected by ELISA in serum samples from (a) 22 patients with cysticercosis, (b) 33 patients with *T. solium* taeniasis, (c) 15 patients with *T. saginata* taeniasis, (d) 59 patients with other parasitoses (schistosomiasis, ancylostomiasis, strongyloidiasis, ascariasis, Chagas' disease, and syphilis), and (e) a group of uninfected subjects. SPA purified by MAbs 2D5C2 (SPA 80), 2D5D9 (SPA 10a), and 1F5C3 (SPA 10b) were used.

LITERATURE CITED


