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Purification of cystic fluid antigen of *Taenia solium* metacestodes by affinity chromatography using monoclonal antibody and its antigenic characterization

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INTRODUCTION

Since Nieto's extensive application of complement fixation test in etiologic differentiation of neurocysticercosis from other neurologic diseases in 1956, many serologic techniques have been successively applied for the differential diagnosis (Flisser and Larralde, 1986). These techniques include precipitin reaction, indirect hemagglutination test, gel diffusion techniques, indirect fluorescent antibody test, intradermal test, enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay. In many cases of the neurocysticercosis, serologic tests were very helpful for a correct diagnosis because they confirmed or supplemented the clinical diagnosis obtained by brain computerized tomography.

Although various serological techniques have been evaluated, these tests still have many problems to be improved. There are some false negative responses in proven neurocysticercosis patients and conversely, a varying degree of false positive reactions in cases of proven other diseases or in normal. Particularly a certain degree of cross reactions with other parasitic infections presented difficulties.

In the past, most workers have used crude extracts of parenchymal tissue of either adult *Taenia solium* or its metacestodes as antigens in the serologic tests (Flisser and Larralde, 1986; Choi *et al.*, 1986). Recently, however, cystic fluid (CF) of *T. solium* metacestodes was found to be more sensitive and specific as a diagnostic antigen than the parenchymal antigens which were prepared from scolex, bladder wall of the metacestodes or adult *T. solium*/*T. saginata*(Choi *et al.*, 1986). CF is now regarded as the most useful antigen in detecting specific IgG antibody in cases of neurocysticercosis by ELISA(Cho *et al.*, 1986; Larralde *et al.*, 1986).

Even with CF, however, cross reactions were found to occur with other parasitic infections although the frequency has been decreased. In an effort to overcome such cross reactions, purer antigens have been searched for. So far, so-called antigen B of Flisser *et al.* (1980) has been the most sensitive and specific among purified antigens. Antigen B makes a precipitating line with 84% of positive sera by immunoelectrophoresis(IEP) and also reacts most frequently with 86% of sera of immunized mice (Yakoleff-Greenhouse *et al.*, 1982). Guerra *et al.* (1982) purified it from crude antigen and found it to be composed of 2 polypeptides of 105k and 95k daltons. Espinoza *et al.* (1982) used it as a diagnostic antigen in ELISA and reported the sensitivity to be 83% in neurocysticercosis. Employing chromatofocusing procedure, Coker-Vann *et al.* (1984) purified antigen of *T. solium*

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metacestodes, whose protein composition appeared to be very complex.

It is now known that antibody responses to *T. solium* metacestodes in man is heterogeneous. Flisser *et al.* (1980) found by IEP that at least 8 precipitating lines were formed with patients' sera; and any class of immunoglobulin of the specific antibody was produced. Recently Grogl *et al.* (1985) observed antibody responses in neurocysticercosis patients by western blot and found that out of 51 recognized bands at least 31 different proteins reacted to the patients' sera.

These findings indicate the necessity to improve the quality of the diagnostic antigen by a new approach to overcome the difficulty of cross reactivity in diagnostic serology. Thus, an attempt is made in the present investigation to purify CF antigen by affinity chromatography using monoclonal antibody (McAb) and to determine the degree of improvement in antigenicity by the process.

MATERIALS AND METHODS

1. Antigens

A total of 7 different helminthic antigens of 6 species was prepared. CF antigen of *T. solium* metacestodes was obtained as described by Choi *et al.* (1986). The protein content in CF was 5.0 mg/ml as measured by method of Lowry *et al.* (1951). Saline extract of parenchymal tissue (scolex and bladder wall) of *T. solium* metacestodes (SBE) and that of adult *T. saginata* (TsWWE) were prepared as Choi *et al.* (1986) described. Saline extract of plerocercoid larva of *Spirometra mansoni* (sparganum) (SprWWE) was prepared by the method described by Kim *et al.* (1984). Hydatid cystic fluid (HCF) was purchased from Tulloch and Co., San Antonio, Texas, USA. Saline extracts of adult *Paragonimus westermani* (PwWWE) was made as described by Cho *et al.* (1981). Saline extract of adult *Clonorchis sinensis* (CsWWE) was also prepared using 8-week old adults collected from an experimental rabbit.

2. Immunization

A total of 10 female BALB/c mice of 6-week old was immunized with CF of *T. solium* metacestodes. They were injected intraperitoneally with 0.1 ml of CF (protein amount 0.1 mg) mixed in 0.1 ml of Freund's complete adjuvant. Six weeks after, 0.1 mg of antigen mixed in Freund's incomplete adjuvant was injected. Ten weeks after the first immunization, the third immunization was made with 0.1 mg of antigen. On 3 days after the third injection, specific IgG antibody levels in sera of immunized mice were checked by ELISA. Three weeks after the third immunization, 0.1 ml of CF was injected intravenously.

On 3 days after the last injection, the mice spleens were removed aseptically, and teased in Dulbecco's Modified Eagle's Medium (DMEM, Flow) to release spleen cells. Mixed red blood cells were hemolysed in Tris-NH_4Cl solution (Tris 20.6 g/l, NH_4Cl 8.3 g/l). Then the number of spleen cells was adjusted to 10^7/ml of DMEM. These spleen cells were used in hybridization.

3. Culture of mouse plasmacytoma and process of hybridization

The mouse plasmacytoma used in cell hybridization was P3×63Ag8.653 which lacked hypoxanthine guanine phosphoribosyl transferase and did not produce any immunoglobulin. The cell line was maintained in DMEM supplemented with 20 μg/ml of 8-azaguanine, 20% fetal bovine serum and 20 μg/ml of gentamycin.

Cells were hybridized according to the method of Cha *et al.* (1984) which was a modification of Köhler and Milstein (1975). The mixture of 10^8 spleen cells and 10^7 plasmacytoma cells was washed 3 times with DMEM by centrifuge. One ml of 50% (w/w) polyethyleneglycol 1000 (Sigma) was added drop by drop to the cells pellets during 1 minute at 37°C, then 15 ml of DMEM were added during next 15 minutes to maintain the hybridization.

After the processes of hybridization, cells were floated in DMEM which contained 20% of fetal bovine serum and 10% medium NCTC-

135 (Gibco); aliquots of 50 μl of the cell suspension were dispensed to 96-well culture plates. In 5—10% CO_2 incubator at 37°C, selective cultivation of the hybridized cells were performed in HAT medium (DMEM with 20% fetal bovine serum, 10% NCTC-135 medium, 20μg/ml of gentamycin, 13.6μg/ml of hypoxanthine, 0.174 μg/ml of aminopterine and 3.87μg/ml of thymidine). On the 1st, 3rd, and 5th day of cultivation, HAT medium was added to the wells. The growth of hybrid cell colonies was monitored daily on inverted microscope.

4. Antigen-binding specificity of antibodies from hybrid cell colonies

To select the antibody-secreting hybrid cell out of grown colonies, culture media in each well were screened by ELISA on 10-14 days of culture. The hybrid cell colonies which secreted the specific IgG antibody were transferred to 24-well plates with HT media which contained all HAT components except aminopterine. The culture media of hybrid cells were examined for their antigen-binding specificity against 7 helminthes antigens.

5. Cell cloning and antigen-binding specificity of monoclonal antibody

The antibody-secreting hybrid cells were diluted in 100 μl of HT media so as the number of cells in a well to be 0.1—1.0 (limiting dilution). They were cultured for 10—14 days in HT media.

When the diameter of a colony from a cloned cell reached 0.5mm, the culture media were screened for the presence of the antibody by ELISA. The antibody-secreting cloned cells were expanded in 24-well plates. Their culture media were examined again for antigen-binding specificity against 7 different helminthic antigens.

In addition, IgG subclasses of the McAb were determined by sandwich-ELISA as described by Cha *et al.* (1984). Wells in polyvinyl plates were coated with anti-mouse IgG_1, IgG_{2a}, IgG_{2b} and IgG_3(Miles). After reacting culture media, peroxidase conjugated rabbit anti-mouse IgG was reacted.

6. Mass production of monoclonal antibody *in vivo*

Following intraperitoneal injection of 0.5ml of pristane (2, 6, 10, 14-tetramethylpentadecane, Sigma), female BALB/c mice were inoculated in peritoneum with 2×10^7 cloned cells. It took about 10—14 days for mice to be full of ascites.

Collected ascites was centrifuged; its supernatant was freezed at −70°C until use. McAb in ascites was purified by the method of Hudson and Hay (1980). The ascites was diluted in physiological saline (1 : 2); it was precipitated with saturated $(NH_4)_2SO_4$ in 45% (v/v). Precipitate was washed once with 45% $(NH_4)_2SO_4$; then with 40% solution. The precipitate was diluted in small amount of phosphate buffered saline (PBS, pH 7.2), dialysed in PBS and in coupling buffer in sequence.

7. Preparation of immunoadsorbent sepharose 4B

A total of 50mg of McAb was mixed with 10 ml of CNBr-activated sepharose 4B(Pharmacia), added with coupling buffer (0.1M $NaHCO_3$, 0.5M NaCl, pH 8.3), and shaken for 2 hours at room temperature. After coupling, protein concentration in the supernatant was measured to calculate the amount of coupled McAb. Forty-eight mg of immunoglobulin of 50mg was coupled.

The uncoupled active sites of CNBr-activated sepharose 4B were blocked by 1M ethanolamine for 2 hours at room temperature. Coupled sepharose 4B was washed 3 times with coupling buffer (pH 8.3), and acetate buffer (0.1M acetate, 0.5M NaCl, pH 4.0) alternately then washed finally with PBS (pH 7.2).

8. Affinity chromatography of cystic fluid antigen through immunoadsorbent coupled with monoclonal antibody

Affinity chromatography was done as described by Hudson and Hay (1980). Column of 1.6× 5 cm of McAb-coupled immunoadsorbent was prepared in equilibrium with PBS. A total of 5 ml of CF (protein amount 25mg) was charged to the column at a flow rate of 10 ml/hour. Elution of unbound protein was monitored by

UV-monitor (LKB) until the absorbance (abs.) was lowered under 0.02. The fractions of unbound protein were pooled. After washing the column with PBS, desorption buffer (0.1M glycine, HCl, pH 2.5) was applied to elute out the bound protein to the McAb. Immediately after pooling of eluted fractions, pH of medium was adjusted to 8.5 by titrating Tris solution and the protein solution was dialysed in PBS.

9. Observation of protein composition by polyacrylamide gel electrophoresis (disc-PAGE)

Disc-PAGE was done as described by Davis (1964) and Ornstein (1964). The protein concentrations of samples were adjusted to 50 μg/100 μl. Electrophoresis was done using 7.5% separating gel columns of 5×100 mm. DC power was supplied at 1.5 mA/gel for 30 minutes and 3 mA/gel for 2 hours. The gel was stained with 0.25% Coomassie brilliant blue R-250 (Sigma) for 2 hours and destained with 10% methanol and 10% acetic acid. The protein composition was traced with densitometer (Gelman) at 565 nm.

10. Characterization of the affinity-purified antigen

The sensitivity of the affinity-purified antigen was compared with CF and unbound pool by ELISA in 26 confirmed cases of neurocysticercosis whose sera and cerebrospinal fluid (CSF) were positive for the specific IgG antibody when CF was used as antigen (Cho *et al.*, 1986).

Sera from patients of other parasitic diseases were tested by ELISA to evaluate the specificity of the affinity-purified antigen. These patients included 23 cases of human sparganosis who were diagnosed serologically (Kim *et al.*, 1984), 15 cases of confirmed *T. saginata* infection, 19 cases of egg positive paragonimiasis, 30 cases of egg positive clonorchiasis, 29 cases of proven other neurologic diseases and 13 uninfected persons.

11. Measurement of antibody levels by ELISA

(1) Measurement in culture media

Wells of polystyrene plate(EIA Plate, Costar) were coated with 50 μl of 7 different antigens in a protein concentration of 5.0 μg/ml overnight at 4°C. After washing 3 times with PBS/Tween 20, 50 μl of culture media were reacted for 2 hours at 37°C. After washing, 50 μl of 1 : 2,000 diluted peroxidase conjugated rabbit anti-mouse IgG (heavy and light chain specific, Cappel) were allowed to react for 2 hours at 37°C. After washing, 50 μl of substrate (1 ml of 1% *o*-phenylene diamine, 10 μl of 30% H_2O_2, 99ml of d.w.) were reacted for 30 minutes at room temperature. The reaction was stopped by adding 50 μl of 4N H_2SO_4. The abs. was read at 492 nm using ELISA reader and corrected by the abs. of reference sera of anti-mouse IgG.

(2) Measurement in human sera and CSF

The specific IgG antibody levels in human sera and CSF were measured by ELISA as described by Cho *et al.* (1986). The protein concentration of respective antigens in coating the plate was 2.5 μg/ml.

RESULTS

1. Specific antibody levels in mice immunized with cystic fluid

On 3 days after the third immunization, blood was collected from ophthalmic venous plexus. Specific IgG antibody levels were measured in sera (Fig. 1). In all mice, the antibody titers

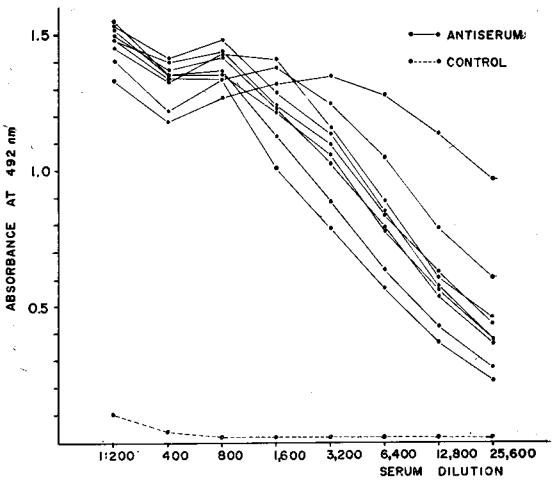

Fig. 1. Titration of sera from 10 immunized BALB/c mice for CF-specific IgG antibody by ELISA.

Table 1. Frequency of wells with growing hybrid cells in HAT media after cell hybridization and those with secreting antibodies reacting to CF of *T. solium* metacestodes

	Frequency(%)
Wells dispensed with cell mixture	792
Wells with growing hybrid cells in HAT medium	299(37.8%)
Wells with hybrids secreting antibodies in culture medium*	71 (9.0%)

* Determined by ELISA (Abs. 0.2 or higher was regarded positive)

were over 1:25,600.

2. Selective culture of hybridoma cells and screening for antibody-secreting colonies

After the processes of hybridization, cells in 792 wells were selectively grown up in HAT media for 10—14 days. Of them, 299 wells (37.8%) had one or more grown colonies. When the culture media were screened for CF-specific IgG antibody, 71 wells(9.0%) were found to secrete the antibody (Table 1).

Cells in 71 wells were amplified in HT media using 24-well culture plates. The culture media were screened again for antigen-binding specificity (Table 2). The highest level of antibody was abs. below 1.0. In Table 2, abs. to each antigen was shown as scores (0-9) which were decimal steps of abs. Antibody levels of score 2 or higher were regarded as positive. By the criterion, a total of 11 combinations antigen-binding specificity was observed.

Group I, which secreted antibody reacting exclusively to CF, was the most frequent (39/71 wells, 54.9%). Nineteen wells (26.8%) of Group II secreted the antibody reacting to both CF and SBE of *T. solium* metacestodes. Three wells (4.2%) were in Group III which was reacting to both CF and TsWWE. Two wells in respect were in Group IV (reacting to CF, SBE and TsWWE) and in Group V (to CF, TsWWE and CsWWE). One well in respect was observed in Groups VI-XI which showed different antigen-binding specificity to a antigen, to a combination of antigens or non-reacting.

3. Antigen-binding specificity of monoclonal antibodies

Except a well in Group XI in which no specific antibody secretion was confirmed, a well

Table 2. Antigen-binding specificity of antibodies in culture supernatant of 71 wells with hybrid cells in HT medium as determined by ELISA using 7 different helminthes antigens

Group of hybrids	No. of wells (%)	Reactivities (as shown by mean score*) to antigen** of						
		CF	SBE	TsWWE	SprWWE	HCF	PwWWE	CsWWE
I	39 (54.9)	3	1	1	1	1	1	1
II	19 (26.8)	4	2	1	1	1	1	1
III	3 (4.2)	2	1	2	1	1	1	1
IV	2 (2.8)	5	2	2	1	1	1	1
V	2 (2.8)	3	1	2	1	1	1	2
VI	1 (1.4)	2	1	1	1	1	1	3
VII	1 (1.4)	3	2	1	1	2	1	2
VIII	1 (1.4)	4	2	2	2	2	2	2
IX	1 (1.4)	1	5	1	1	1	1	1
X	1 (1.4)	1	1	2	1	1	1	1
XI	1 (1.4)	1	1	1	1	1	1	1

* Mean score 0: abs. 0.00~0.10, 1: abs. 0.11~0.20, 2: abs. 0.21~0.30, 3: abs. 0.31~0.40, 4: abs. 0.41~0.50, 5: abs. 0.51~0.60, 6: abs. 0.61~0.70, 7: abs. 0.71~0.80, 8: abs. 0.81~0.90, 9: abs. 0.91~1.00.

** CF: Cystic fluid of *T. solium* metacestodes, SBE: Scolex and bladder wall extract (parenchymal extract) of *T. solium* metacestodes, TsWWE: Saline extract of adult *T. saginata*, SprWWE: Saline extract of sparganum, HCF: Cystic fluid of hydatid, PwWWE: Saline extract of adult *Paragonimus westermani*, CsWWE: Saline extract of adult *Clonorchis sinensis*

Table 3. Antigen-binding specificity and subclass of immunoglobulin G of monoclonal antibodies secreted by 83 hybridoma lines

Cell line secreting McAb	Origin of cell (Group)	No. of wells (%)	Reactivities (as shown by mean score*) to antigen							Sub-class** of McAb
			CF	SBE	TsWWE	SprWWE	HCF	PwWWE	CsWWE	
CFCc-143	I	24	5	1	1	1	1	1	1	IgG_1
CFCc-12A	IV	4	5	1	1	1	1	1	1	IgG_1
CFCc-117	V	2	2	1	1	1	1	1	1	IgG_1
CFCc-71A	IX	1	2	1	1	1	1	1	1	not IgG
subtotal		31 (37.3%)								
CFCc-298	II	37	7	2	1	1	1	1	1	IgG_1
CFCc-12B	IV	1	5	2	1	1	1	1	1	IgG_1
CFCc-17A	VIII	7	3	2	1	1	1	1	1	not IgG
subtotal		45 (54.2%)								
CFCc-12C	IV	1	2	1	2	1	1	1	1	IgG_1
CFCc-17B	VIII	2	3	2	1	1	1	1	2	not IgG
CFCc-71B	IX	1	1	5	1	1	1	1	1	IgG_1
CFCc-150	VI	3	1	1	1	1	1	1	1	IgG_1

* Mean score: same as in Table 2 ** as tested by sandwich-ELISA

in each Group was selected for cloning. As a criterion of selection, the antibody titer in culture media was considered. A total of 192 wells was inoculated with cloned cell. Out of them 83 wells showed growth of one colony each. Of them, all but 2 colonies secreted CF-specific IgG antibody.

Cloned hybridoma lines in 83 wells were expanded in 24-well culture plates. Culture media were screened again for antibody specificity against 7 antigens (Table 3). Out of 83 wells, 31 (37.3%) secreted McAb reacting only to CF. Although these 31 lines originated from colonies of Groups I, IV, V and IX, most of them came from Group I (24/31).

Cloned cell lines which secreted McAb reacting to both CF and SBE originated from Groups II, IV and VIII (45/83 wells, 54.2%), most of them coming from Group II (37/45).

Other cell lines that secreted McAb reacting to different combinations of antigen were as follows: One clone that reacted to CF and TsWWE, 2 clones that reacted to CF, SBE and CsWWE, one clone that reacted to only SBE.

McAb secreted by 8 cloned hybridoma lines belonged to subclass IgG_1 out of 11 tested. Immunoglobulins secreted by 3 remaining lines were not IgG.

4. Mass production of monoclonal antibody *in vivo*

A hybridoma cell line, coded as CFCc-298.18, that secreted McAb reacting to CF in the highest titer (score 7) and to SBE in a low positive range (score 2) was selected for mass production. From 18 ml of mice ascites, 143 mg (of protein) of McAb was obtained by $(NH_4)_2SO_4$ precipitation.

5. Affinity chromatography of cystic fluid by immunoadsorbent column coupled with monoclonal antibody

When 5 ml of CF was applied to the column, 2 fractions (2.5 ml/fraction) were eluted without any significant abs. by UV-spectrophotometry. Then a large peak, reaching the highest abs. to 2.0, appeared. These fractions were pooled and designated as unbound pool of cystic fluid antigen (U-Ag).

After washing the column with PBS, the bound protein to McAb was eluted by desorption buffer. Another peak appeared. These fractions were pooled and named as an affinity-purified antigen (A-Ag). When desorption buffer containing 10% dioxane, was applied to the column again, a minor peak (abs. up to 0.1) appeared. The fraction was pooled and named as an affinity-purified minor fraction (a-Ag) (Fig. 2).

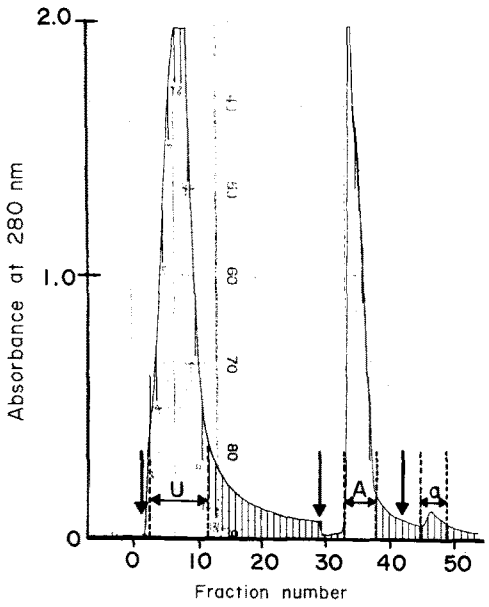

Fig. 2. Profile of affinity chromatography of CF of *T. solium* metacestodes using immunoadsorbent of CFCc-298.18 McAb. Unbound protein(U) was washed away with PBS (first arrow). The first population of bound protein molecules(A) was eluted with 0.1M glycine-HCl buffer (pH 2.5) (2nd arrow), the second population (a) was eluted with same buffer containing 10% dioxane (3rd arrow).

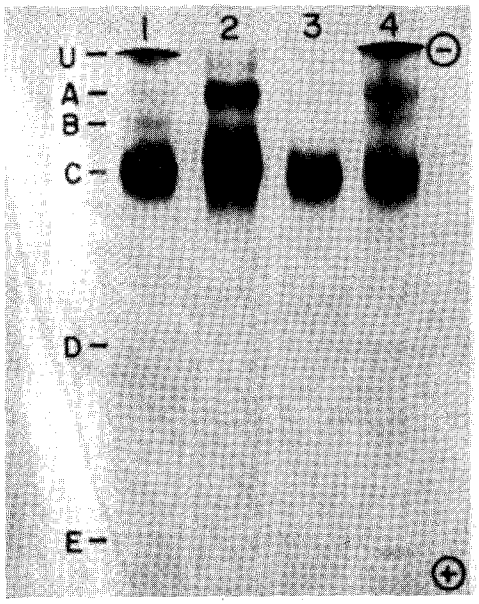

Fig. 3. Banding patterns of proteins in crude and purified antigens by disc-PAGE. 1: CF, cystic fluid of *T. solium* metacestodes, 2: A-Ag, affinity-purified pool (1st run) from CF, 3: A-Ag, affinity-purified pool (2nd run) from CF, 4: U-Ag, pool of unbound proteins from affinity chromatography of CF.

The yield of A-Ag was calculated by measuring the protein concentrations in respective pools of fractions (Table 4). From 25 mg of protein in CF, 5.5 mg (22.0%) was recovered as A-Ag. When once used column was charged again with 45 mg of protein in CF, 3.8 mg of A-Ag (8.4%) was obtained. Total yield of A-Ag was 1.2 mg when U-Ag was applied to the used column for the second time.

6. Protein composition of fractionated proteins

Banding patterns of A-Ag, U-Ag and CF in disc-PAGE were shown in Fig. 3. The densitometric tracing of the banded proteins were shown in Table 5.

CF was composed of 6 protein bands; of them band C was 69.1% of total and band U was 13.9%. In A-Ag (lst run), 3 bands were recognized. Of them, 2 bands were identical with bands A and C of CF respectively: Their protein were 18.7% and 78.0% of total respectively. Another small band of A-Ag near band

Table 4. Yields of fractionated antigen by affinity chromatography using CFCc-298.18 McAb

Run	Amount of protein (% yield) of			
	applied CF	unbound pool(U-Ag)	affinity pool(A-Ag)	affinity pool(a-Ag)
first	25 mg	12.5 mg	5.5 mg (22 %)	0.9 mg
second	45 mg	— *	3.8 mg (8.4%)	—
third	unbound pool 2nd run	—	1.2 mg	—

* not checked

Table 5. Relative composition of protein bands in disc-PAGE of crude and affinity-purified antigens of CF of *T. solium* metacestodes as measured by densitometric tracing

Band	Relative proportion (%) in			
	CF	A-Ag (1st run)	A-Ag (2nd run)	U-Ag
U	13.9	3.3*	0	13.9
A	6.8	18.7	7.0	(30.2)
B	9.7	0	0	
C	69.1	78.0	93.0	55.6
D	0.1	0	0	0.2
E	0.5	0	0	0.1

* located between band U and band A

U was not recognized in disc-PAGE of either CF or U-Ag. The percentage of protein in A-Ag (2nd run) was 7.0% of band A and 93.0% of band C.

In U-Ag, all of 6 protein bands in CF were identified. Comparing with CF, however, the relative composition of band C decreased from 69.1% of CF to 55.6% of U-Ag.

7. Antigenic characteristics of affinity-purified antigen

(1) Sensitivity

In Fig. 4, the results of ELISA were presented by abs. using CF, A-Ag and U-Ag as antigens. The sera and CSF from 26 confirmed neurocysticercosis were positive by CF. If the same abs. 0.18 is regarded as a positive criterion in other two antigens, 7 cases of 26(26.9%) by sera and 8 cases (30.8%) of 26 by CSF turned to negative when A-Ag was used. When U-Ag was used, only one case by CSF turned to negative. Accordingly, the sensitivity of A-Ag was calculated as 73.1% of CF when sera were tested and 69.2% when CSF were tested.

(2) Levels of antibody (Abs.) detected by affinity-purified antigen

The mean abs. by ELISA in sera and CSF were 1.00 and 0.98 in respect when CF was used as antigen. Meanwhile, the mean abs. decreased to 0.49 in sera and 0.58 in CSF when the same concentration of protein of A-Ag was used in ELISA. In U-Ag, mean abs. were 1.06 and 1.06 in respect (Fig. 4).

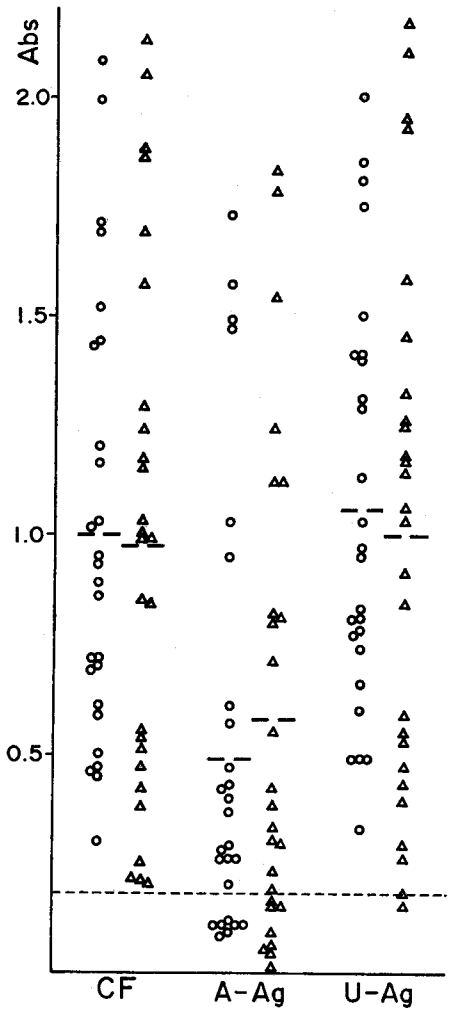

Fig. 4. Scattergram of absorbance by ELISA in sera (○) and CSF (△) of confirmed neurocysticercosis patients using crude and purified antigens. CF: Cystic fluid of *T. solium* metacestodes, A-Ag: Affinity-purified pool of CF (1st rnn), U-Ag: Unbound pool from affinity chromatography.

As shown in Fig. 5, the individual abs. in sera and CSF were highly correlated when the respective antigens were used.

(3) Specificity

In Table 6, the results by ELISA using 3 antigens were presented is sera from other parasitic infections, other neurologic diseases and normal control. Cross reactions were observed in 5 of 129 cases when CF was used

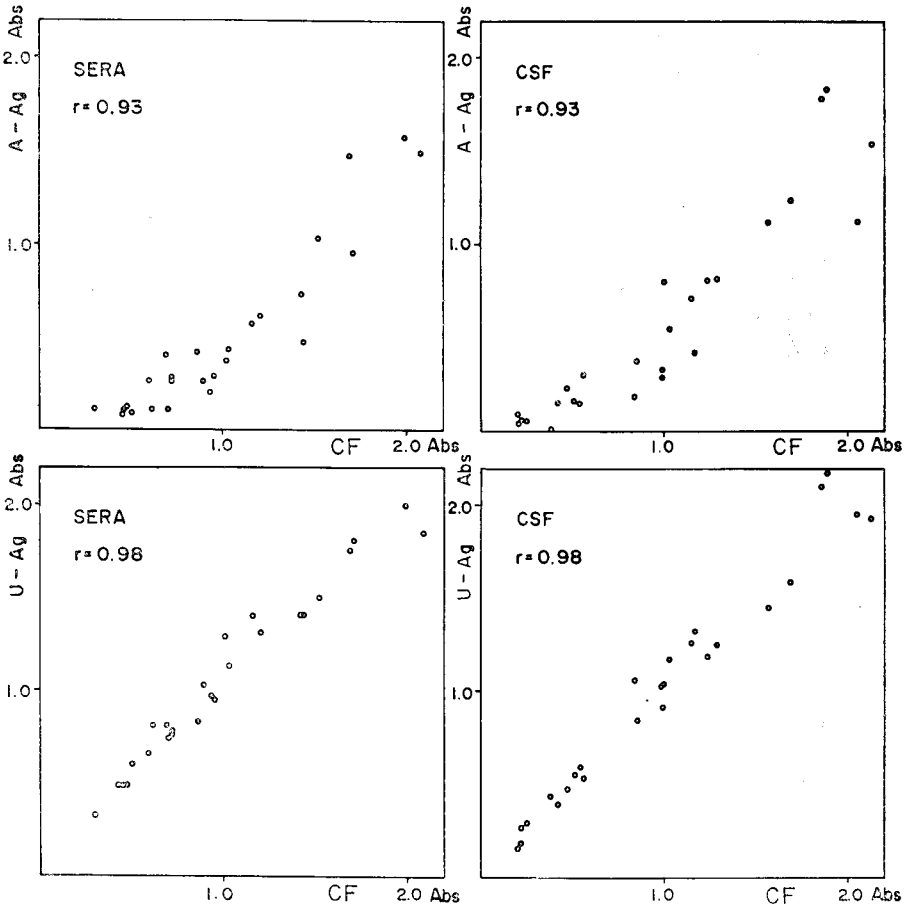

Fig. 5. Relations of abs. by ELISA in sera and CSF of confirmed neurocysticercosis patients using crude and purified antigens.

Table 6. Specificity of crude and purified antigens of *T. solium* metacestodes as determined by ELISA with sera from other helminthic infections, other neurologic diseases and normal control

Category	No. of cases	No. (%) of positive* cases using		
		CF	U-Ag	A-Ag
Sparganosis	23	2 (8.7)	4 (17.4)	0
Taenia saginata infection	15	2 (13.3)	3 (20.0)	0
Paragonimiasis	19	1 (5.3)	1 (5.3)	0
Clonorchiasis	30	0	0	0
Other neurologic diseases	29	0	0	0
Normal control	13	0	0	0

* Positive criterion: abs. 0.18 or higher in all tests

as antigen while those were found in 8 cases when U-Ag was used. However, no cross reaction was observed when A-Ag was used.

The individual abs. of the cross-reacted cases were shown in Table 7. Except a case of sparganosis, abs. in all cases decreased below 0.09 when A-Ag was used.

Table 7. Absorbance of sera in cross-reacted patients against 3 antigens as measured by ELISA

Category	Our serum No.	Absorbance of sera against		
		CF	U-Ag	A-Ag
Sparganosis	205	0.15	0.18	0.02
	489	0.30	0.31	0.04
	789	1.02	1.25	0.17
	1,153	0.14	0.18	0.09
	Mean	0.40	0.48	0.08
Taenia saginata	263	0.22	0.19	0.02
infection	569	0.19	0.20	0.04
	729	0.15	0.10	0.04
	Mean	0.19	0.19	0.04
Paragonimiasis	45	0.18	0.19	0.05

DISCUSSION

The occurrence of cross reactions between antigen of *T. solium* metacestodes and sera from other parasitic infections or *vice versa* indicated the presence of common antigenic determinants in relevant parasites. There have been many examples of parasitic infections in which serologic cross reactions occurred with cysticercosis: Hydatid disease (Rydzewski *et al.*, 1975; Schantz *et al.*, 1980; Diwan *et al.*, 1982), sparganosis (Kim *et al.*, 1984; Cho *et al.*, 1986), adult *Taenia* infections (Proctor *et al.*, 1966; Mahajan *et al.*, 1974; Cho *et al.*, 1986), schistosomiasis (Diwan *et al.*, 1982), clonorchiasis, paragonimiasis, fascioliasis (Cho *et al.*, 1986) and angiostrongyliasis (Diwan *et al.*, 1982).

At present, a few molecular characteristics of common antigenic determinants which are responsible for such cross reactions are known. McAb as produced by the method of Köhler and Milstein (1975) is now regarded as a powerful probe solving the questions concerning the common antigenic determinants (Mitchell, 1982). Especially in complex antigens of parasites, affinity chromatography using McAb would provide many experimental data on the cross reactions.

In the present experiment, mice were immunized with CF of *T. solium* metacestodes and the antigen-binding specificities of antibodies secreted in each well with colonies of hybrid cells were examined. By the screening, the colonies that secreted antibody reacting only to CF were the most frequent (Group I in Table 2). It indicated that plasma cells in spleen of BALB/c mice responded most frequently to the antigenic determinant in components unique to CF.

The colonies that secreted antibody reacting to both CF and SBE (Group II) were the second in frequency. It may suggest that parenchymal components are also present in CF. At this stage, however, it was not clear whether the colonies were composed of hybrid cells secreting antibody that reacted to both antigens or mixture of different hybrid cells that secreted antibody reacting to only one antigen. The presence of immunogens unique only to CF and common to both CF and SBE had been described in a series of reports by Flisser *et al.* (1975, 1979, 1980). When double immunodiffusion was done using scolex, bladder wall and CF antigens and their respective antisera, a line specific to CF was found along with common lines between them. They also confirmed these findings by IEP. Choi *et al.* (1986) observed the disc-PAGE patterns of scolex, bladder wall and CF antigens; a protein band, band C was the most abundant in CF. In addition, they found protein bands common in 3 antigens. All these findings supported that in CF there are not only a specific antigenic protein but also some common components.

The hybridoma colonies that secreted antibody reacting to 7 screened antigens in combination or independently (Groups IV-XI in Table 2) also indicated that the common antigenic determinants were present in CF. Unlike the relatively frequent occurrence of cross reaction between human cysticercosis and sparganosis/hydatid disease, mice hybrids reacted to SprWWE or HCF very rarely. This difference may be interpreted in two ways. One was the different attitudes between mouse and human in recognizing the complex immunogens. Another possible

explanation was that the antigenic determinants eliciting the cross reactions in human were not present in CF of *T. solium* metacestode.

After the cloning the hybrid colonies, all of cloned hybridoma from Groups I, II and IX showed the same antigen-binding specificity with those before cloning. However, cloned hybridoma from colonies of Group IV, VIII and IX secreted antibodies reacting only to a part of the previously reacted antigens. This means that the colonies before cloning was polyclonal. Some of cloned hybridoma stopped the antibody secretion after a certain time.

Out of the hybridomas we made, the one which secreted antibody reactive to both CF (abs. 0.75) and SBE (abs. 0.25) was chosen for affinity-chromatography, rather than that which secreted antibody reacting only to CF. In affinity-chromatography, the adhesive ability as a ligand was considered to be more important than the presence of weak cross reactivity. Furthermore, affinity-chromatography using the McAb was expected to purify only one protein which was common in both CF and SBE. Unlike the expectation, however, affinity-purified antigen of CF was found to be composed of 3 protein bands by disc-PAGE. Out of them, one was never recognized in disc-PAGE of CF or U-Ag; it was considered to be an artifact probably appeared during acidic process of desorption. Therefore, A-Ag was made of 2 bands of proteins, band A and band C. Band A was a common component protein present both in CF and SBE (Choi *et al.*, 1986). In the second run of immunoadsorbent column, the obtained A-Ag had a reduced amount of band A than in the first. It was probably due to the priority of band C over band A in competitive binding to the McAb in reduced state of active ligands of the second run. The present result also suggested that the common antigenic determinant that reacted to the McAb was located not only in band C protein but also in band A protein of CF components. This needs confirmation.

The reactivity of McAb to antigenic proteins of known molecular weight should be investigated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western-blot methods. In addition, the relations of A-Ag with the already known, purified antigens should also be clarified. As for the purified antigen B of Flisser *et al.* (1980), the protein molecule was found to be made of 2 polypeptides having molecular weights of 105k and 95k daltons each (Guerra *et al.*, 1982). These two polypeptides were reported to have same antigenicity and to be glycoprotein containing 12% of carbohydrates. Grogl *et al.* (1985) described that 51 protein bands were recognized in SBE of *T. solium* metacestodes by SDS-PAGE. Out of them, 31 bands reacted specifically with sera from 5 neurocysticercosis patients by western-blot. The major antigenic proteins were 200k, 64k, 62/61k, 53k, 45k, 41k, 36/35k, 30k and 16k daltons. They could not recognize the antigen B component. These conflicting results concerning the specific antigenic proteins of *T. solium* metacestodes awaits for further investigations.

When compared to CF and U-Ag, A-Ag was less sensitive than CF and U-Ag. This suggested that BALB/c mice recognized the immunogenic protein especially the antigenic determinants in band C protein far more frequently than human patients did. Another plausible explanation for lower abs. and sensitivity was that serologic test using A-Ag detected only a single or limited numbers of monospecific antibodies reacting to antigenic determinants in band A and band C out of diverse polyclonal antibodies in patient serum.

A-Ag, of which main component was band C, showed higher specificity than CF or U-Ag did. Cross reactions, observed in the latter two antigens, disappeared when A-Ag was used. Accordingly, A-Ag was considered to be useful in differentiation of causative agent, in cross-reacting patients such as sparganosis and paragonimiasis. U-Ag in which the relative amount of band C was reduced, was as valuable as CF with regard to the sensitivity and specificity. This indicated that polyclonal antibodies were produced in cysicercosis patients specific not only

to band C but also to many other immunogenic components of parasite proteins.

SUMMARY

This study was undertaken to purify cystic fluid (CF) antigen of *Taenia solium* metacestodes by affinity chromatogaphy using specific monoclonal antibody(McAb) and to characterize the antigenicity of the purified antigen.

The hybridoma cell lines, prepared by fusion between mouse plasmacytoma and spleen cells from BALB/c mice immunized with CF, secreted antibodies reacting to various helminthic antigens. Majority of cell lines reacted to CF only but some also reacted to parenchymal antigen of *T. solium* metacestodes, adult *T. saginata*, sparganum, hydatid cystic fluid, *Paragonimus westermani* and *Clonorchis sinensis*, either in combination with CF, other antigens or independently.

Cloned cells derived from monoclonal lines also produced antibodies reacting either to CF only or to other helminthes in combination or independently. These results indicated that CF of *T. solium* metacestodes contained proteins which possessed antigenic determinants not only specific to CF but also cross reactive with the afore-mentioned helminthes.

CF of *T. solium* metacestodes was purified by affinity chromatography using the McAb which reacted to CF and parenchymal antigens. The affinity-purified antigen (A-Ag) and unbound pool CF (U-Ag) were separated. A-Ag showed 2 protein bands by disc-PAGE whereas CF exhibited 6 bands and U-Ag consisted of all bands CF had.

The diagnostic significance of A-Ag was evaluated by ELISA in human neurocysticercosis and other helminthic and neurologic diseases. By A-Ag, the levels of the specific IgG antibody, as shown by absorbance in sera and CSF, were lower than those of CF and U-Ag. Accordingly, the sensitivity was about 70% of CF and U-Ag. However, the nonspecific positive reactions to CF and U-Ag, observed in sparganosis, *T. saginata* infection and paragonimiasis did not occur when A-Ag was used.

These results indicated that the affinity-purified A-Ag had the higher specificity but the lower sensitivity as a diagnostic antigen in cysticercosis, probably because it only detected a single or limited numbers of monospecific antibodies among the diverse polyclonal antibodies produced in the patients with neurocysticercosis.

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=국문요약=

단세포군항체에 의한 유구낭미충 낭액 특이항원의 순수분리 및 항원특성 관찰

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이 연구는 특이 단세포군항체를 이용하여 친화 크로마토그래피를 실시함으로써 유구낭미충의 낭액에 특이한 항원을 순수분리하고 분리한 항원의 진단용 항원으로서의 특성을 관찰하고자 실시하였다.

유구낭미충 낭액을 BALB/c 마우스에 면역시켜 얻은 비장세포와 마우스 형질세포종을 융합하여 얻은 하이브리도마세포가 분비하는 항체의 항원 결합특이성을 면역효소측정법으로 먼저 관찰하였다. 하이브리도마 세포는 대부분(54.9%) 유구낭미충 낭액에만 반응하는 항체를 생산하였다. 그러나 낭액항원과 기타 기생충항원에 같이 반응하는 항체 또는 기타 기생충항원 한가지에만 반응하는 항체등을 분비하는 하이브리도마세포 집락도 있었다.

무한대 희석법으로 하이브리도마세포를 희석 분주하여 단세포배양을 하였다. 이 경우에도 배양액에 분비한 단세포군 항체는 낭액항원에만 반응하는 경우에 대부분이었으나 기타 기생충 항원에 반응하는 단세포군 항체도 얻을 수 있었다. 따라서 유구낭미충 낭액에는 낭액에 특이한 항원결정기 이외에 유구낭미충의 두절 및 낭벽항원, 무구조충 및 간흡충등과 같은 항원결정기도 갖고 있음을 알 수 있었다.

단세포군 항체중 낭액항원과 가장 높은 항원항체 반응을 일으키며 두절 및 낭벽항원에도 약한 반응을 보였던 단세포군 항체를 선택하고 이를 이용하여 친화 크로마토그래피를 시행하였다. 낭액항원은 순수분리항원(A-Ag) 및 단세포군 항체와 결합하지 않았던 단백질의 총합(U-Ag)으로 분리하였다. 폴리아크릴아마이드 젤 전기영동법으로 낭액항원, A-Ag 및 U-Ag의 단백질 조성을 관찰한 바 낭액항원과 U-Ag는 6개의 band로 되어 있었으나 A-Ag은 band A 및 band C 두가지로 구성되어 있고 그중 대부분은 band C이었다.

순수분리한 A-Ag의 진단용 항원으로서의 가치를 면역효소측정법으로 관찰하였다. 낭액항원을 이용하여 혈청 및 뇌척수액내 IgG 항체가가 양성이었던 뇌유구낭미충증 환자에 대하여 A-Ag(같은 단백질함량)를 항원으로 면역효소측정법을 실시한 바 민감도는 낭액항원이나 U-Ag에 비하여 70%로 낮아졌다. 그러나 낭액항원 및 U-Ag이 무구조충 감염자, 스파르가눔증 환자 및 폐흡충증 환자에 대해 나타내는 교차반응이 A-Ag를 항원으로 사용하였을 때에는 모두 사라졌다.

이와같은 결과에서 단세포군 항체를 친화 크로마토그래피의 반응고리로 사용하여 순수분리한 항원은 유구낭미충 낭액항원중 특이한 항원결정기를 갖는 단백질로 구성되어 있음을 알 수 있었다. 그러나 순수분리항원은 유구낭미충증 환자의 혈청 및 뇌척수액에 존재하는 다세포기원 항체중 A-Ag의 특이항원결정기에만 반응하는 단세포군 항체 하나 또는 몇가지와만 반응하게 함으로써 특이성은 높아지나 혈청학적 민감도는 저하하였다고 판단하였다.