기 생 충 학 잡 지 제25권 제2호
The Korean Journal of Parasitology
Vol. 25, No. 2, 159-167, December 1987

Analysis of antigen specificity using monoclonal and polyclonal antibodies to *Cysticercus cellulosae* by enzyme-linked immunoelectrotransfer blot technique*

Seung-Yull Cho, Shin-Yong Kang and Suk-Il Kim
Department of Parasitology, College of Medicine, Chung-Ang University, Seoul 151, Korea

INTRODUCTION

One of the problems met in serologic diagnosis of cysticercosis is cross reactions with other parasitic diseases. Even by sensitive method of enzyme-linked immunosorbent assay(ELISA), patients of schistosomiasis, echinococcosis, angiostrongyliasis, taeniasis, sparganosis, paragonimiasis, clonorchiasis and fascioliasis may show cross reactions with crude antigens from *Taenia solium* metacestode(*Cysticercus cellulosae*) (Arambulo *et al.*, 1978; Diwan *et al.*, 1982; Coker-Vann, *et al.*, 1984; Mohammad *et al.*, 1984; Cho *et al.*, 1986). To solve the problem, thus to improve the test specificity, better antigens with the least cross reactivity and with high sensitivity have been searched for (Biagi and Tay, 1958). Recently cystic fluid of *C. cellulosae* was found to be the case (Choi *et al.*, 1986; Larralde *et al.*, 1986). However, cystic fluid antigen also elicited a certain degree of non-specific reactions (Cho *et al.*, 1986). Therefore, purification of antigen by biochemical or immunochemical procedures has been considered to be essential in improving the quality of antigen (Guerra *et al.*, 1982; Coker-Vann *et al.*, 1984; Kim *et al.*, 1986).

Since electrophoretic transfer of proteins in gel to nitrocellulose paper was developed (Towbin *et al.*, 1979), enzyme-linked immunoelectrotransfer blot (EITB, western blot) was formulated (Tsang *et al.*, 1983). When sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmi, 1970) is applied in EITB, protein fractions of known molecular weight in crude antigens can be observed whether they are actually immunogenic. Therefore, EITB is especially applicable in varieties of parasitic infections to differentiate the antigenic proteins and to observe their antigenic specificities. EITB is now considered a key step in solving the problems of cross reactions in serologic diagnosis of parasitic diseases (Tsang *et al.*, 1983).

In human cysticercosis, results of EITB have already been reported several times (Grogl *et al.*, 1985; Gottstein *et al.*, 1986; Larralde *et al.*, 1986; Joo *et al.*, 1987). However, their results were not necessarily identical. Some uncertainties are still exist. Furthermore, hitherto reported results of EITB were reactions between patients sera from various parasitic diseases and crude *C. cellulosae* antigens.

To analyse antigen specificity of differently prepared crude antigens of *C. cellulosae* and other helminthic antigens with polyclonal and a monoclonal antibody, SDS-PAGE and EITB were done.

MATERIALS AND METHODS

1. Antigens

Three different antigens from *C. cellulosae*

* This study was supported by grant from the Ministry of Education, Republic of Korea(1986).

were prepared as described by Choi *et al.* (1986): (1) cystic vesicular fluid antigen which contained 5mg/ml of protein as measured by Lowry *et al.* (1951), (2) saline extract of tissue of scolices of *C. cellulosae* and (3) saline extract of whole worm of *C. cellulosae* including cystic fluid.

In addition, saline extract of larval sparganum (Kim *et al.*, 1984), *Paragonimus westermani* (Cho *et al.*, 1981), *Clonorchis sinensis* and *Fasciola* sp. (Cho *et al.*, 1986) were also used. Hydatid cyst fluid was collected from a Korean patient returned from Saudi Arabia.

2. Antibodies

A monoclonal antibody against cystic fluid of *C. cellulosae* as prepared by Kim *et al.* (1986) was used.

A total of 24 sera from confirmed neurocysticercosis patients was used as polyclonal antibodies. Only serum from each patient was tested. The patients were diagnosed on the bases which were described by Cho *et al.* (1986): *i.e.*, characteristic brain computerized tomographic findings, clinical symptoms of seizure, headache, and neurologic symptoms such as aphasia, hemiparesis *etc.* and positive serologic test by ELISA either in serum or in cerebrospinal fluid or in both. Four of 24 patients were surgically confirmed as neurocysticercosis while other 6 patients were by biopsy of subcutaneous nodules.

By ELISA, absorbance in sera from 24 patients were 0.20~1.18 (mean abs. 0.75, standard deviation, 0.30) to cystic fluid antigen.

3. SDS-PAGE

All SDS-PAGE chemicals including marker proteins were obtained from Sigma Chemicals Co. (USA). Vertical electrophoresis system of 17 ×12cm was used. Separating polyacrylamide gel of about 9cm long and 1.5mm thick was prepared in 1.5M Tris buffer (pH 8.8) containing 0.4% SDS. Linear gradient gel of 10~15% were used because 8% or 15% gels did not separate all proteins, especially of low molecular weight (data not shown). Stacking gel of about 1cm long was 3% polyacrylamide in 0.5M Tris buffer (pH6.8) containing 0.4% SDS. Different antigens were adjusted to contain 1~2mg/ml of protein as measured by Lowry *et al.* (1951) after lyophilization and rehydration. Antigens were treated at 95°C for 5 minutes with same amount of sample buffer (0.125M Tris, pH 6.8 containing 20% glycerol, 4.5% SDS, 10% mercaptoethanol, and 0.01% bromphenol blue). Thirty μl of antigens (containing 30~60 μg of protein) were applied to each well. Samples were electrophoresed at 30mA for 3~4hours. Protein bands were stained overnight with 0.125% Coomassie brilliant blue R-250 containing 4.6% acetic acid and 25% methanol. Finally it was destained with 25% methanol and 10% acetic acid solution.

4. EITB

The methods described by Tsang *et al.* (1983) were adopted. Resolved protein fractions by SDS-PAGE were transferred to nitrocellulose paper (Bio-Rad) in Towbin buffer containing 20% methanol. Electrophoresis was done at 100V for 2 hours in a refrigerator. After electrophoresis the nitrocellulose paper was washed on shaker 3 times with phosphate buffered saline (pH 7.4)/0.5% Tween 20 (PBS/T) each for 5 minutes. Then 100ml of 1 : 100 diluted monoclonal antibody or patient sera were reacted on shaker at room temperature for 1 hour. After washing with PBS/T 3 times again, 100ml of 1 : 2,000 diluted conjugates (peroxidase conjugated anti-human IgG goat IgG for patients sera and peroxidase conjugated anti-mouse IgG rabbit IgG for monoclonal antibody, heavy- and light-chain specific, Cappel, USA) were reacted for 1 hour. After washing, substrate (50mg of 3,3′-diaminobenzidine, 10μl of 30% H_2O_2 and 100ml of distilled water) was reacted for 15 minutes. The reaction was stopped by washing the paper with distilled water for 3 times.

RESULTS

1. SDS-PAGE

As shown in Fig. 1, each antigen was separated into many bands by SDS-PAGE. In cystic fluid of *C. cellulosae* (lane 3), at least 23 bands

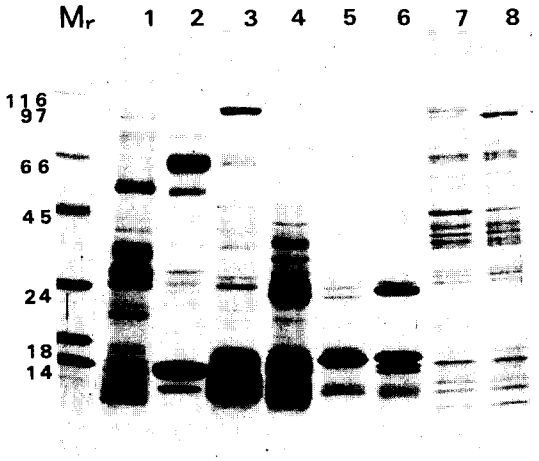

Fig. 1. SDS-PAGE of 8 antigens in 10~15% linear gradient gel, stained with 0.125% Coomassie brilliant blue R-250. M_r: Standard protein markers, 1: saline extract of sparganum, 2: hydatid cyst fluid, 3: cystic fluid of *C. cellulosae*, 4: saline extract of *Fasciola*, 5: saline extract of *Clonorchis*, 6: saline extract of *Paragonimus*, 7: saline extract of scolex of *C. cellulosae* and 8: saline extract of whole worm of *C. cellulosae*.

were recognized (7 kilodalton (kDa)->200kDa). Of them, 9 bands of 94, 64, 48, 39, 34, 24, 15, 10 and 7 kDa were major bands. Especially, 15, 10 and 7kDa bands constituted 70% of total protein as measured by densitometry (data not shown).

Saline extract of scolex of *C. cellulosae* showed more bands (at least 34) than cystic fluid (lane 8). Ten protein bands of 94, 64, 39, 34, 26, 24, 21, 15, 10 and 7 kDa were common with bands in cystic fluid, but their concentration may be different. Faintly stained bands of molecular weight above 70 kDa were more numerous in saline extract of scolex than in cystic fluid. Saline extract of whole *C. cellulosae* worms showed identical protein bands with scolex extract, but their stainability may be different by band(lane 9).

Saline extract of sparganum (lane 1) showed at least 28 recognizable protein bands in SDS-PAGE. Of them, 87, 78, 53, 40, 34, 29, 26, 23, 21, 15, 9 and 6 kDa were major bands. Hydatid cystic fluid (lane 2) revealed 15 bands. Of them, 64, 52, 29, 27, 14, 8 kDa were major bands.

Saline extract of adult *Fasciola*(lane 4) showed at least 22 bands in SDS-PAGE. Of them, 38, 35, 32, 29, 25 and 17-6 kDa bands were major bands. Saline extract of *C. sinensis* showed 6 bands(lane 5). Of them, 15 and 8 kDa were major bands. Saline extract of *P. westermani* showed 11 bands(lane 4). Of them, 26, 15,

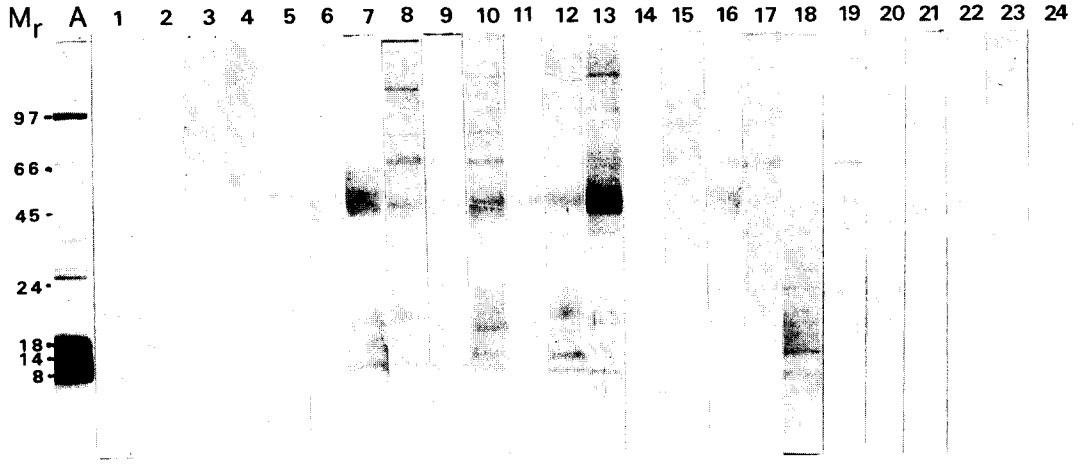

Fig. 2. SDS-PAGE/EITB findings in 24 patients sera of neurocysticercosis to cystic fluid of *C. cellulosae*. M_r: molecular weight in kDa, A: SDS-PAGE of cystic fluid (lane 3 in Fig. 1), 1~24: sera from patients.

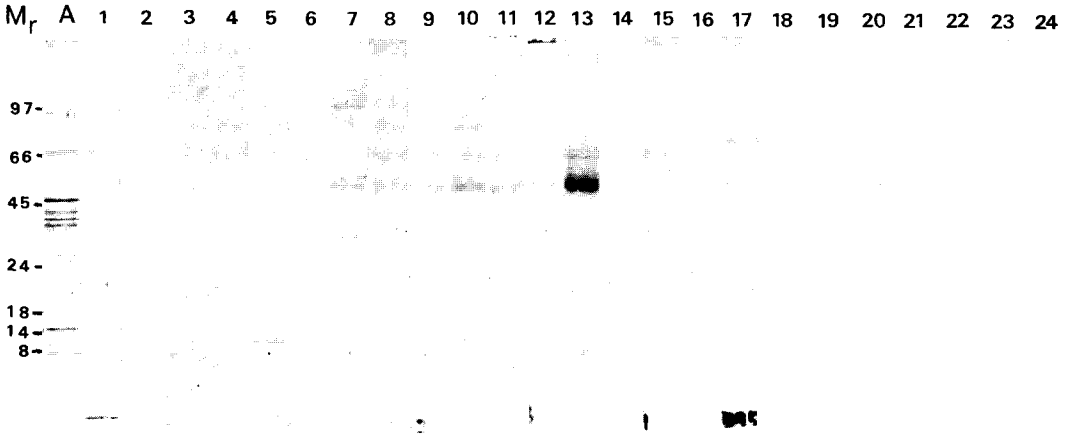

Fig. 3. SDS-PAGE/EITB findings in 24 patients sera of neurocysticercosis to saline extract of scolex of *C. cellulosae*. M_r: molecular weight in kDa, A: SDS-PAGE of the saline extract (lane 7, Fig. 1), 1~24: patients sera.

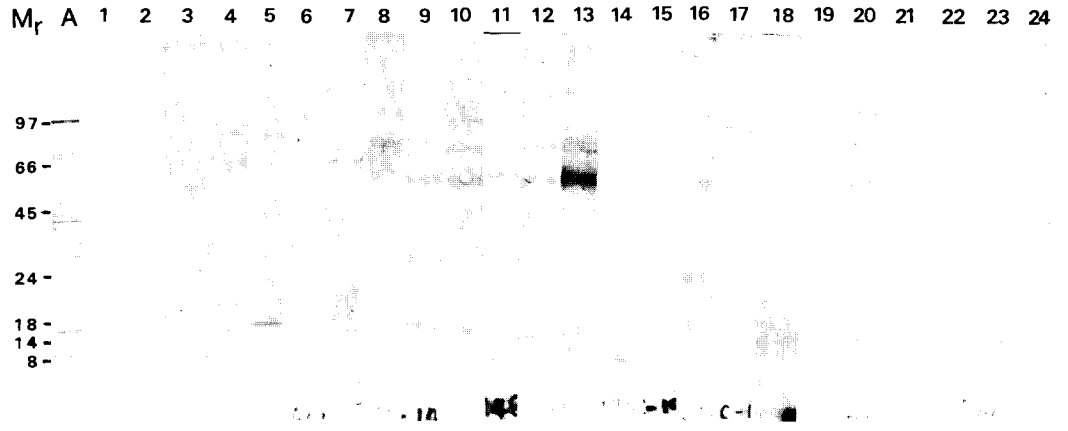

Fig. 4. SDS-PAGE/EITB findings in 24 patients sera of neurocysticercosis to saline extract of whole worm of *C. cellulosae*. M_r: molecular weight in kDa, A: SDS-PAGE of the saline extract (lane 8, Fig. 1), 1~24: patients sera.

13, and 7.5 kDa were major bands.

2. EITB with polyclonal antibody from neurocysticercosis patients

(1) Cystic fluid of *C. cellulosae*

As shown in Fig. 2, all of 24 patients sera reacted with 2-14 bands of cystic fluid (average 7.4 bands/patient). Deeply stained bands in SDS-PAGE did not necessarily react with patient sera. Of 16 reacted bands, 152kDa (13/24 patients), 94kDa (14/24), 72kDa (10/24), 64 kDa (22/24), 48kDa (21/24), 24kDa (12/24), 15kDa (17/24), 10kDa (17/24) and 7kDa (13/24) were frequently reacted bands. Bands of 170kDa (7/24), 120kDa (3/24), 43kDa (3/24), 39kDa (8/24), 34kDa (3/24), 27kDa (1/24) and 22kDa (7/24) were also antigenic.

(2) Saline extracts of scolex and whole worm of *C. cellulosae*

Two antigenic preparations showed almost identical EITB results (Figs. 3 and 4). Out of 24 patients sera, 20 reacted with 1-11 bands (average 6.3 bands). Out of 34 bands in SDS-

PAGE, 16 reacted with one or more patients sera. Of them, 94kDa (13/24 patients), 64kDa (17/24), 52kDa (14/24), 39kDa (13/24,) 34kDa (13/24), 15kDa (11/24) and 10kDa (14/24) were major bands which reacted with patients sera. Bands of 158kDa (4/24), 120kDa (2/24), 75kDa (6/24), 57kDa (5/24), 46kDa (6/24), 27kDa (4/24), 24Da (8/24), 20kDa (2/24) and 7kDa (9/24) were also antigenic. Especially, faint stained band of 52kDa in SDS-PAGE were found to be a highly antigenic by EITB.

(3) Saline extract of sparganum

Out of 24 patients sera, 17 showed reactions with 1-8 protein bands (average 2.7) of sparganum extract (Fig. 5). Out of 28 bands in SDS-PAGE, 12 may react with patient sera. Of them, 130kDa (11/24 patients) and 64kDa (12/24) were most frequently reacted.

Bands of 158kDa (7/24), 143kDa (3/24), 87 kDa (7/24), 78kDa (7/24), 72kDa (2/24), 53 kDa (8/24), 29kDa (3/24), 26kDa (1/24) and 21kDa (1/24) may react crossly with polyclonal antibody from neurocysticercosis patients.

(4) Hydatid cyst fluid

Out of 24 patients sera, 13 showed cross reactions with 1-4 bands in SDS-PAGE of hydatid

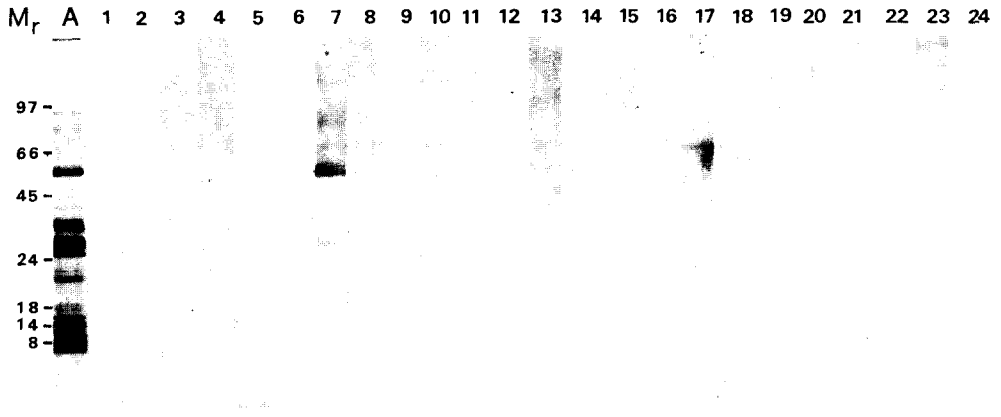

Fig. 5. SDS-PAGE/EITB findings in 24 patients sera of neurocysticercosis to saline extract of sparganum. M_r: molecular weight in kDa, A: SDS-PAGE of the saline extract (lane 1 in Fig. 1), 1~24: patients sera.

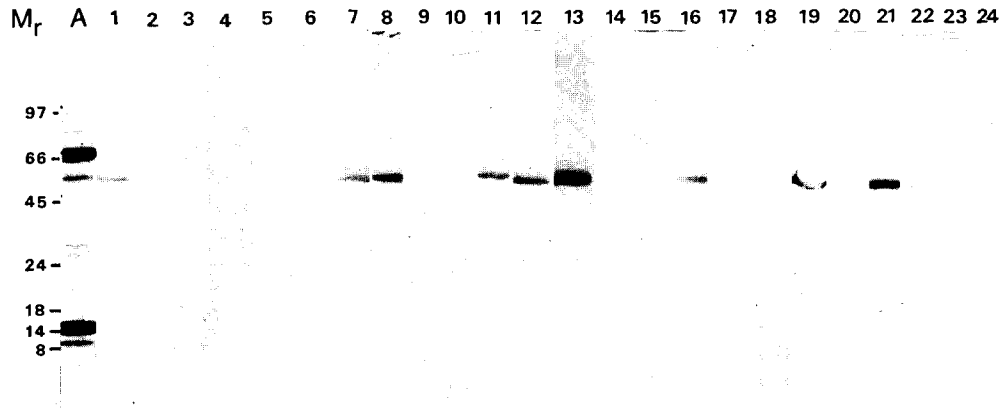

Fig. 6. SDS-PAGE/EITB findings in 24 patients sera of neurocysticercosis to hydatid cyst fluid. M_r: molecular weight in kDa, A: SDS-PAGE of hydatid cyst fluid (lane 2 in Fig. 1), 1~24: patient sera.

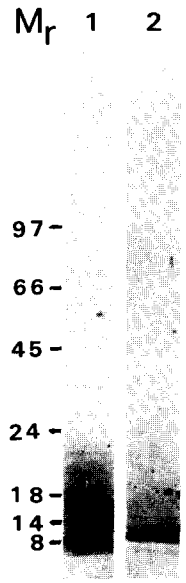

Fig. 7. SDS-PAGE/EITB findings of a monoclonal antibody to antigens of *C. cellulosae*. M_r: molecular weight in kDa, 1: cystic fluid, 2: saline extract of scolex.

cyst fluid (average 1/patient) (Fig. 6). Of 15 bands recognized in SDS-PAGE, 6 may show cross reactions with cysticercosis sera. Of them, 52kDa (12/24 patients) and 27kDa (5/24) were most frequently reacted. Bands of 64kDa (1/24), 29kDa (2/24), 10kDa (1/24) and 8kDa (1/24) may also react with the polyclonal antibody.

(5) Saline extracts of *Fasciola*, *Clonorchis* and *Paragonimus*

These 3 extracts did not show cross reactions with the polyclonal antibody from 24 neurocysticercosis patients (data not shown).

3. EITB with monoclonal antibody

Out of the monoclonal antibodies prepared by Kim *et al.* (1986), we selected CCH 298. 18 to evaluate its antigenic specificity in SDS-PAGE. As shown in Fig. 7 protein bands of 15, 10 and 7kDa reacted with the antibody. In addition, a faint reaction was observed at band of 34kDa. In saline extract of scolex of *C. cellulosae*, a band of 10kDa was reacted with the antibody. Other extracts did not show any reaction.

DISCUSSION

Different results of the western blot have been reported concerning specific protein antigens in *C. cellulosae*. Grogl *et al.* (1985) reported that 9 protein bands of 200, 64, 62/61, 53, 45, 41, 36/35, 30 and 16kDa were major protein bands which reacted with sera from 5 neurocysticercosis patients. Gottstein *et al.* (1986) reported that out of many protein bands, 26 and 8kDa were polypeptides specific to *C. cellulosae* when cross reacting components with sera from other parasitic diseases were excluded. Larralde *et al.* (1986) described that 103kDa band elicited the strongest antibody response in neurocysticercosis patients. Joo *et al.* (1987) concluded that bands of 91, 63 and 21kDa in cystic fluid and 62, 43 and 14kDa in parenchymal antigen of *C. cellulosae* were the proteins specifically reacting with sera from cysticercosis patients.

Besides minor differences in calculating the molecular weight of protein bands in SDS-PAGE, confusions on the specific proteins as revealed by EITB may be arisen from (1) differently prepared antigens, (2) different technique of SDS-PAGE especially separating gel concentration, (3) limited number of tested patients sera and (4) difficulties in defining the status of individual patients.

SDS-PAGE in this study revealed that cystic fluid was different from parenchymal antigen in its relative concentration rather than composition of proteins. In cystic fluid, proteins of low molecular weight (15, 10 and 7kDa) were found to be major components. So far these low molecular weight proteins in SDS-PAGE were neglected in previous reports except that by Gottstein *et al.* (1986). More importantly, by EITB, these proteins were found to be major bands which react with over 2/3 of tested patients.

Results of EITB concerning protein bands specific to *C. cellulosae*, so far, were too complicated to draw any definite conclusion. However, strongly reacting protein bands of 94, 64 and

52kDa in either cystic fluid or parenchymal antigen were cross reacting components. High molecular weight bands of proteins above 100 kDa as seen in SDS-PAGE seems also common antigens between parasites as shown by Gottstein *et al.* (1986). In addition, sera from cysticercosis patients reacted with high molecular weight bands in sparganum extract.

As seen in Figs. 2, 3 and 4 in this study, individual patient revealed different reactions to different antigen bands. Therefore, results of SDS-PAGE/EITB in each patient may reflect a stage of infection. If the origin of each protein band in SDS-PAGE is known, together with the sequence of antigenic stimulations, during long course of the disease, serologic staging of a patient of cysticercosis would be possible. Because our monoclonal antibody reacted to low molecular proteins of 15, 10 and 7kDa, the purified antigen by immunoaffinity chromatography by Kim *et al.* (1986) should be identical protein. The protein was less sensitive but more specific than crude cystic fluid antigen in the diagnosis (Kim *et al.*, 1986). Its usefulness as a staging antigen deserves further study.

SUMMARY

To analyse the antigen specificity of patients sera from 24 confirmed neurocysticercosis and a monoclonal antibody, SDS-PAGE using 10∼15% linear gradient gel and EITB were done. Cystic fluid, saline extracts of scolex and of whole worm of *C. cellulosae*, saline extracts of sparganum, hydatid cyst fluid, saline extracts of *Fasciola*, *Clonorchis* and *Paragonimus* were used as antigen.

Of protein bands in cystic fluid of *C. cellulosae*, patient sera reacted frequently to bands of 152, 94, 64, 48, 24, 15, 10 and 7kDa proteins. To saline extracts of scolex and whole worm of *C. cellulosae*, patients sera reacted frequently to 94, 64, 52, 39, 34, 15 and 10kDa bands. Two bands in sparganum extract (130 and 64kDa) and two bands in hydatid cyst fluid (52 and 27kDa) were cross-reacting bands with sera from cysticercosis patients. Saline extracts of *Fasciola*, *Clonorchis* and *Paragonimus* did not exhibit cross-reacting bands. Monoclonal antibody to cystic fluid of *C. cellulosae* was found to react with low molecular weight proteins of 15, 10 and 7kDa.

ACKNOWLEDGEMENTS

The authors thank to Prof. H.S. Lee, Department of Biochemistry, Chung-Ang University (CAU), Assoc. Prof. S.C. Park, Dept. of Biochemistry, Seoul National University and Dr. I.H. Kim, Dept. of Biochemistry, Dong-A University for their encouragement and advices in setting SDS-PAGE and EITB. Miss H.S. Kang, Department of Parasitology, CAU and Mr. Chi Young Park, a medical student of CAU helped the laboratory procedures.

REFERENCES

Arambulo, P.V., Walls, K., Bullock, S. and Kagan, I.G. (1978) Serodiagnosis of human cysticercosis by micro-plate enzyme-linked immunospecific assay (ELISA). *Acta tropica*, **15**:63-67.

Biagi, F. and Tay, J. (1958) A precipitation reaction for the diagnosis of cysticercosis. *Am. J. Trop. Med. Hyg.*, **7**:63-65.

Cho, S.Y., Hong, S.T., Rho, Y.H., Choi, S. and Han, Y.C. (1981) Application of micro-ELISA in serodiagnosis of human paragonimiasis. *Korean J. Parasit.*, **19**(2):151-156.

Cho, S.Y., Kim, S.I., Kang, S.Y., Choi, D.Y., Suk, J.S., Choi, K.S., Ha, Y.S., Chung, C.S. and Myung, H. (1986) Evaluation of enzyme-linked immunosorbent assay in serological diagnosis of human neurocysticercosis using paired samples of serum and cerebrospinal fluid. *Korean J. Parasit.*, **24**(1):25-41.

Choi, B.K., Kim, S.I., Kang, S.Y. and Cho, S.Y. (1986) Evaluation of antigens from different parts of *Cysticercus cellulosae* in serological diagnosis of human cysticercosis. *Chung-Ang J. Med.*, **11**(2):135-146.

Coker-Vann, M., Brown, P., Gadjusek, D.C. (1984) Serodiagnosis of human cysticercosis using a chromatofocused antigenic preparation of *Taenia*

solium cysticerci in an enzyme-linked immunosorbent assay (ELISA). *Trans. Roy. Soc. Trop. Med. Hyg.*, 78:492-496.

Diwan, A.R., Coker-Vann, M., Brown, P., Subianto, D.B., Yolken, R., Desowitz, R., Escobar, A., Gibbs, C.J. and Gadjusek, D.C. (1982) Enzyme-linked immunosorbent assay (ELISA) for the detection of antibody to cysticerci of *Taenia solium*. *Am. J. Trop. Med. Hyg.*, 31:364-369.

Gottstein, B., Tsang, V.C.W. and Schantz, P.M. (1986) Demonstration of species specific and cross-reactive components of *Taenia solium* metacestode antigens. *Am. J. Trop. Med. Hyg.*, 35(2):308-313.

Grogl, M., Estrada, J.J., MacDonald, G. and Kuhn, R.E. (1985) Antigen-antibody analyses in neurocysticercosis. *J. Parasit.*, 71(4):433-442.

Guerra, G., Flisser, A., Canedo, L. and Laclette, J.P. (1982) Biochemical and immunological characterization of antigen B purified from cysticerci of *Taenia solium*. In: *Cysticercosis: Present status of knowledge and perspectives* (Ed.: Flisser, A. *et al.*), 437-451, Academic Press, New York.

Joo, K.H., Kang, S.H., Lee, J.S. and Rim, H.J. (1987) Studies on the demonstration of species specific and cross reactive components of vesicular fluid and parenchymal antigens from *Cysticercus cellulosae*. *Korea Univ. Med. J.*, 24(3):139-150.

Kim, H., Kim, S.I. and Cho, S.Y. (1984) Serological diagnosis of human sparganosis by means of micro-ELISA. *Korean J. Parasit.*, 22(2):222-228.

Kim, S.I., Kang, S.Y., Cho, S.Y., Hwang, E.S. and Cha, C.Y. (1986) Purification of cystic fluid antigen of *Taenia solium* metacestodes by affinity chromatography using monoclonal antibody and its antigenic characterization. *Korean J. Parasit.*, 24(2):145-158.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of Baceriophage T_4. *Nature*, 227:681-685.

Larralde, C., Laclette, J.P., Owen, C.S., Madrazo, I., Sandoval, M., Bojalil, R., Sciutto, E., Contreras, L., Arzate, J., Diaz, M.L., Govezensky, T., Montoya, R.M. and Goodsaid, F. (1986) Reliable serology of *Taenia solium* cysticercosis with antigens from cyst vesicular fluid: ELISA and hemagglutination tests. *Am. J. Trop. Med. Hyg.*, 35(5):965-973.

Lowry, O.H., Rosenbrough, N.J., Lewis-Farr, A. and Randall R.J. (1951) Protein measurement with Folin-phenol reagent. *J. Biol. Chem.*, 193:265-275.

Mohammad, I.N., Heiner, D.C., Miller, B.L., Goldberg, M.A. and Kagan, I.G. (1984) Enzyme-linked immunosorbent assay for the diagnosis of cerebral cysticercosis. *J. Clin. Microbiol.*, 20(4):775-779.

Towbin, H., Staehelin, T. and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci.*, 76:4350-4354.

Tsang, V.C.W., Peralta, J.M. and Simons, A.R. (1983) Enzyme-linked immunoelectrotransfer blot techniques (EITB) for studying the specificities of antigens and antibodies separated by gel electrophoresis. *Meth. Enzymol.*, 92:377-391.

=國文抄錄=

酵素免疫電氣泳動移滴法을 利用한 有鉤嚢尾蟲 單細胞群抗體 및 患者血淸에 對한 抗原特異性 分析

中央大學校 醫科大學 寄生蟲學敎室
趙 昇 烈·姜 信 榮·金 錫 一

寄生蟲疾患의 診斷에도 利用하는 血淸學的 診斷에서는 近緣寄生蟲抗原과 交叉反應이 나타나 特異度를 떨어뜨리는 境遇가 있어 問題가 되고 있다. 診斷用으로 使用하는 寄生蟲粗抗原의 蛋白質 構成成分中 交叉反應을 가장 잘 일으키는 것이 어떤 것인지를 알 수 있다면 特異度가 높은 抗原을 製作하는 基本情報로 使用할 수 있을 것이다. 最近 SDS-폴리아크릴아마이드젤 電氣泳動(SDS-PAGE)과 酵素免疫電氣泳動移滴法(EITB)을 利用하여 抗原抗體反應을 일으키는 蛋白質成分을 粗抗原中에서 區別할 수 있게 되었다. 이 硏究에서는 有鉤嚢尾蟲의 嚢液, 頭節의 生理食鹽水抽出液, 全嚢尾蟲抽出液과 스파르가눔抽出液, 包蟲嚢液, 肝蛭抽出液, 肝吸蟲抽出液, 肺吸蟲抽出液 등 8種의 抗原에 對하여 有鉤嚢尾蟲症 患者 24名의 血淸과 本敎室에서 製作한 單細胞群抗體가 어떻게 反應하는지 觀察하여 抗原特異性을 分析하고자 하였다.

SDS-폴리아크릴아마이드젤 電氣泳動은 길이 9cm, 두께 1.5mm인 10~15% linear gradient gel을 使用하였고 試料 30μl를 sample buffer와 함께 95°C에서 5分間 加熱한 후 3~4시간 電氣泳動하였다. 分離된 蛋白質을 다시 100V에서 2시간 동안 Towbin buffer에서 電氣泳動하여 nitrocellulose 종이로 移滴하였다. 그후 患者血淸(1 : 100), 單細胞群抗體(1 : 100)과 反應시키고 peroxidase-conjugated antihuman IgG(또는 peroxidase-conjugated antimouse IgG), 基質의 順으로 反應시켜 發色하였다. 그 結果를 要約하면 다음과 같다.

1. SDS-폴리아크릴아마이드젤 電氣泳動後 Coomassie brilliant blue R-250으로 染色한 바 有鉤嚢尾蟲 嚢液에는 蛋白質帶가 最小 23個 나타나고 그中 94, 64, 48, 39, 34, 24, 15, 10, 7kDa가 主蛋白質이었다. 頭節抽出液에는 蛋白質帶 34個가 보였고 그中 94, 64, 39, 34, 26, 24, 21, 15, 10, 7kDa가 主蛋白質帶이었다. 全嚢尾蟲抽出液도 頭節抽出液과 同一한 所見을 보였다. 嚢液抗原과 蟲體抽出液間에는 蛋白質 構成成分보다는 構成比의 差異가 심하였다. 스파르가눔 抽出液은 28個, 包蟲嚢液은 15個, 肝蛭抽出液은 22個, 肝吸蟲抽出液은 6個, 肺吸蟲抽出液은 11個의 蛋白質帶를 보였다.

2. 患者血淸 24個를 SDS-PAGE로 分離된 蛋白質帶와 反應시킨 結果, 嚢液抗原에서는 平均 7.4個와 反應하였는 바 그中 152, 94, 72, 64, 48, 24, 15, 10, 7kDa 蛋白質이 가장 重要하였다. 頭節 抽出液과는 平均 6.3個와 反應하였고 그中 94, 64, 52, 39, 34, 15, 10kDa가 重要하였다. 스파르가눔抽出液과는 平均 2.7個가 反應하였고 그中 130, 64kDa가 重要하였으며 包蟲嚢液과는 平均 1個가 反應하였고 52, 27kDa가 重要하였다. 肝蛭, 肝吸蟲 및 肺吸蟲抽出液과는 反應帶가 觀察되지 않았다.

3. 單細胞群抗體는 嚢尾蟲嚢液의 15, 10, 7kDa 및 頭節抽出液 10kDa와 反應하였다.

以上의 結果에서 有鉤嚢尾蟲의 構成蛋白質中 15, 10, 7kDa 등 低分子量蛋白質이 診斷特異性이 높은 分劃으로 判斷하였다.