기 생 충 학 잡 지 제26권 제3호
The Korean Journal of Parasitology
Vol. 26, No. 3, 163~167, September 1988

Antigenic protein fractions reacting with sera of sparganosis patients*

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Abstract: To observe the antigenic protein fractions in saline extract of *Spirometra mansoni* plerocercoid (sparganum), the crude extract was separated in reducing conditions of sodium dodecyl sulfate-polyacrylamide gel electrophoresis(SDS-PAGE). The proteins, transferred by electrophoresis to nitrocellulose paper, were reacted with sera from 15 surgically confirmed sparganosis and 24 cysticercosis patients for immunoblotting. Out of 30 identified protein bands in the extract, bands of 29 and 36 kilodaltons (kDa) were the strongest and the most frequently reacting with specific antibody (IgG) in sparganosis sera. Bands of higher molecular weight also reacted with the sera but their frequency of reactions was lower. Sera of cysticercosis reacted with different protein bands in saline extract of sparganum, but the cross reactions were observed in strong antigenic bands of 29 and 36 kDa.

Key words: *Spirometra mansoni* plerocercoid, sparganum, antigenic proteins, human sparganosis, human cysticercosis, SDS-PAGE/immunoblot

INTRODUCTION

In human sparganosis, serodiagnosis has only limited applicabilities because of its rare incidence. Preoperatively, even a presumptive diagnosis of this larval disease is very much difficult. Despite the limitation, however, serologic test for sparganosis may be useful in certain situations. For example, seroepidemiological studies, etiologic differentiation of sparganosis from other helminthic infections in patients whose surgical pathology specimens presenting uncertainty in morphologic identification of the worm, and cure criterion of surgically treated patients *etc.* are situations in which serodiagnosis is helpful (Kim *et al.*, 1984).

In addition to the above listed applicabilities, serologic test may be used in screening the rarely occurring sparganosis out of a certain category of patients. For example, by routine serologic screening of neurologic patients in Korea, 12 positive cases of cerebral sparganosis were detected in 3 years. Eventually characteristic findings of brain computerized tomography (CT) in these cerebral sparganosis patients were described by Chang *et al.* (1987). In endemic areas of human sparganosis such as East and Southeast Asian countries, routine screening for sparganum-specific IgG antibody by enzyme-linked immunosorbent assay(ELISA) may have significant value in detecting previously underestimated disease of neurosparganosis (Chang *et al.*, 1987).

Though useful, serologic test by ELISA using crude saline extract of sparganum as antigen

* This study was supported by research grant for genetic engineering from Ministry of Education, Republic of Korea (1988).

revealed cross reactions with taeniasis, cysticercosis(Kim *et al.*, 1984) and possibly with hydatidosis respectively. These cestode parasites may have shared common antigenic determinants each other. To overcome this difficulty arising from cross reactivity in serodiagnosis, species specific antigenic components should be searched for.

This study was undertaken to identify the antigenic protein fractions of sparganum which react with polyclonal antibodies in sera of human sparganosis.

MATERIALS AND METHODS

1. Antigen

Saline extract of sparganum was prepared as described by Kim *et al.*(1984). Briefly, naturally infected spargana in snake, *Natrix tigrina lateralis*, collected in Kangwon Do (Cho *et al.*, 1982), were ground with teflon-coated tissue homogenizer. After adding saline, the emulsion was shaken for 2 hours at room temperature, and kept in a refrigerator for 24 hours. Finally it was centrifuged by 10,000 *g* for 30 minutes at 4°C. The supernatant was regarded as antigen. The protein content in the extract was 5.0 mg/ml as measured by Lowry *et al.* (1951).

2. Patient sera

(1) Sera from sparganosis patients

From August 1984 to January 1988, a total of 90 positive reactors for sparganum-specific IgG antibody have been detected in the Department of Parasitology, Chung-Ang University. Of these, sera of 15 patients were selected in whom sparganosis was surgically confirmed. The antibody titers (absorbance) in these sera were in a range of 0.43～1.05 when tested by ELISA (positive criterion, 0.22) (Kim *et al.*, 1984).

(2) Sera of cysticercosis patients

A total of 24 patients sera of neurocysticercosis was used. All patients were confirmed as cysticercosis by two or more of the following criteria; (1) surgical removal of *Taenia solium* metacestodes from cranial cavity, (2) biopsy of *T. solium* metacestode in subcutaneous tissue, (3) brain CT findings compatible with neurocysticercosis of multiple low densities and (4) positive reaction for cysticercus-specific antibody (IgG) test in serum and cerebrospinal fluid(Cho *et al.*, 1986).

Eight of 24 patients cross-reacted by ELISA with sparganum extract when tested with both antigens. These cross-reacted cysticercosis patients were selected above the normal proportion of such cross-reaction in order to observe the responsible protein components.

3. SDS-PAGE

Methods of Laemmli (1970) were applied. Chemicals including marker proteins were products of Sigma Chemicals (USA). Vertical electrophoresis system of 17×12 cm was used. Separating gel (10～15% linear gradient) of about 9 cm long and 1.5 mm thick contained 0.4% SDS in 1.5M Tris buffer (pH 8.8). Stacking gel of about 1 cm long contained 3% polyacrylamide in 0.5% Tris buffer (pH 6.8) with 0.4% SDS. Saline extract of sparganum was treated at 95°C for 5 minutes with the same amount of sample buffer (0.125M Tris, pH 6.8 containing 20% glycerol, 4.5% SDS, 10% 2-mercaptoethanol and 0.01% bromphenol blue). A total of 50 μl of the prepared crude extract of sparganum was applied to a wide sample well made in the stacking gel. The sample was electrophoresed at 30 mA for 3～4 hours. Protein bands were stained overnight in 0.125% Coomassie brilliant blue R-250 containing 4.5% acetic acid and 25% methanol. Finally it was destained with 25% methanol and 10% acetic acid. When the electrophoresed gel was used in immunoblotting the gel was processed as follows.

4. Immunoblotting

The methods of Tsang *et al.* (1983) were adopted. Resolved protein bands in SDS-PAGE were transferred to nitrocellulose paper in Towbin buffer by electrophoresis at 100 V for 2 hours. After the transfer, nitrocellulose paper was divided longitudinally into 5 mm wide each.

After washing 3 times for 5 minutes each in phosphate buffered saline(pH 7.4)/0.5% Tween 20, 5 ml of 1 : 100 diluted sera of patients were reacted to each strip for 1 hour. After washing, 1 : 2,000 diluted conjugate (peroxidase conjugated anti-human IgG, Cappel, USA) were reacted. Finally, substrate (consisted of 50 mg of 3, 3'-diaminobenzidine, 10 μl of 30% H_2O_2 and 100 ml of distilled water) was reacted for 15 minutes. The reaction was stopped by washing with distilled water 3 times.

RESULTS

1. Protein bands of the antigen as revealed by SDS-PAGE

As shown in Fig. 1, saline extract of sparganum represented 30 stained bands of proteins in SDS-PAGE. The bands were 158, 130, 107, 87, 78, 72, 67, 60, 58, 55, 53, 52, 43, 41, 39, 36, 32, 29, 26, 23, 22, 21.5, 21, 18, 17, 15, 14, 13.5, 9 and 6 kDa respectively. Of them, 87, 78, 52, 36, 29, 23, 21, 15, 14 and 6 kDa bands were stained more darkly and constituted major bands.

2. Reaction patterns of patients' sera

(1) Sera of sparganosis patients

As shown in Fig. 2, sera of sparganosis patients reacted in different combinations to 6~19 protein bands. Among them, 36 and 29 kDa bands exhibited the most frequent reactions (14/15 patients). Patients sera showed concomi-

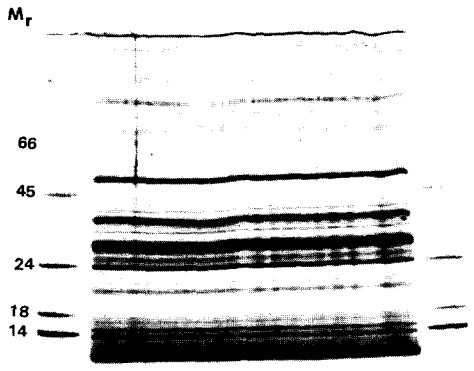

Fig. 1. Findings of SDS-PAGE of saline extract of sparganum. SDS-PAGE was done in 10~15 % linear gradient gel. The saline extract was heat-treated with sample buffer containing 10% 2-mercaptoethanol. Coomassie brilliant blue R-250 stained. M_r: Molecular weight in kDa.

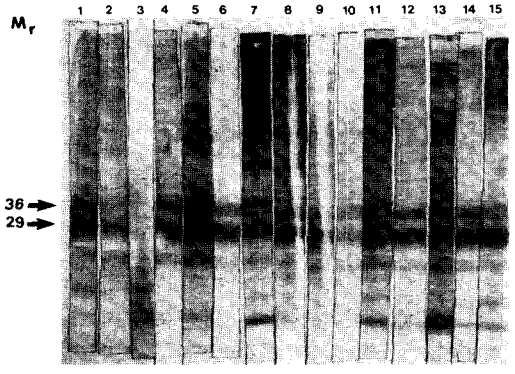

Fig. 2. SDS-PAGE/immunoblot findings in 15 confirmed human sparganosis patients to saline extract of spargana. M_r: Molecular weight in kDa.

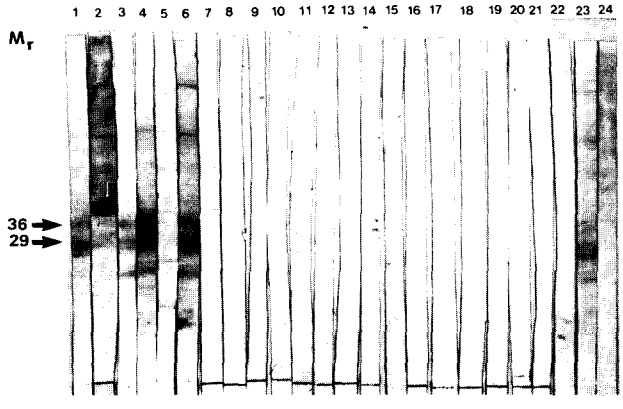

Fig. 3. SDS-PAGE/immunoblot findings in 24 confirmed neurocysticercosis patients to saline extract of spargana. Patients No. 1~6 and 23~24 cross-reacted positively by ELISA against sparganum antigen. M_r: Molecular weight in kDa.

tant positive reactions to the two protein bands. In a patient, concomitant reaction was not observed. Reactions to 29 kDa were stronger in all reacted sera than to 36 kDa. Reactions to 23 kDa band were observed in 13 of 15 patients but reaction was weak. Band of 21 kDa reacted with 3 patients and that of 15 kDa reacted with 6 patients. Band of 6 kDa reacted with 9 patients sera.

Protein bands larger than 36 kDa showed very complicated reactions by patient serum. Bands of 158, 130, 107, 78, 72, 67 and 52 kDa were reacted with patient sera with lower frequency.

(2) Sera of cysticercosis patients

Out of 24 patients of cysticercosis, sera of 10 patients showed reactions with protein bands in sparganum antigen. Strong reactions were observed in 29 and 36 kDa bands especially in 7 of 8 patients whose sera cross-reacted with sparganum antigen by ELISA. Bands of 107, 72, 52 and 21 kDa reacted with sera of cysticercosis in lower frequency (Fig. 3).

DISCUSSION

Serodiagnosis of human sparganosis either by indirect fluorescent antibody test(IFA)(Ishii, 1973) or by ELISA(Kim *et al.*, 1984) has been proved to be sensitive and rather specific. In experimental sparganosis of rabbits, Ohnishi *et al.* (1986) observed that antibody titers by indirect hemagglutination, latex agglutination and IFA increased 1 week after intraperitoneal inoculation. According to them, specific IgG antibody did bind the reticular tissue of sparganum when observed by IFA; and secretory-excretory product of sparganum was a stronger antigen than saline extracts of either sparganum or adult *Spirometra*.

The saline extract of sparganum was separated into 30 bands of protein in reducing SDS-PAGE. Of these bands, it became evident that 29 and 36 kDa bands represented sensitive and strong antigens. Other protein bands were also antigenic as shown in Fig. 2, but their reactions were inconsistent. Cho *et al.* (1987) reported that many protein bands in sparganum antigen may react with sera from cysticercosis patients when examined by immunoblot. Bands of larger molecular weight such as 158, 143, 87, 78 and 52 kDa exhibited more frequent cross reactions. In this study, however, 29 and 36 kDa bands, the strong antigens, cross-reacted with sera from cysticercosis patients. And these cross-reacting sera were positive with crude sparganum antigen by ELISA, while the most of remaining cysticercosis patients were not. Unlike this, sera of human sparganosis rarely cross-reacted with cystic fluid antigen of *T. solium* metacestodes. These results suggest that some human cysticercosis patients may produce specific IgG antibody against antigenic proteins of *T. solium* metacestodes which are common with sparganum. The common antigen may be present in negligible amount in cystic fluid but in large amount in parenchymal portion of the metacestodes. Patients of racemose cysticercosis may be such cases. This should be confirmed in the future.

The nature of 29 and 36kDa proteins in sparganum extract is not known at present. Unlike the above speculation, these protein bands may be components in secretion/excretion from sparganum as Ohnishi *et al.* (1986) observed. In this respect, purified cysteine protease of sparganum was estimated to be 19~21 kDa in molecular weight and its optimum pH was 7.0(Fukase *et al.*, 1985). The relations between protease, the major antigenic proteins identified in this study and secretion/excretion products deserve further studies.

REFERENCES

Chang, K.H., Cho, S.Y., Chi, J.G., Kim, W.S., Han, M.C., Kim, C.W., Myung, H. and Choi, K.S. (1987) Cerebral sparganosis: CT characteristics. *Radiology*, **165**:505-510.

Cho, S.Y., Kang, S.Y. and Kim, S.I.(1987) Analysis of antigen specificity using monoclonal and polyclonal antibodies to *Cysticercus cellulosae* by enzyme-linked immunoelectrotransfer blot tech-

nique. *Korean J. Parasit.*, **25**:159-167.

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 cysticercosis using paired samples of serum and cerebrospinal fluid. *Korean J. Parasit.*, **24**:25-41.

Cho, S.Y., Song, K.W. and Lee, S.H. (1982) Cestode parasites of terrestrial snakes in Korea. *Chung-Ang J. Med.*, **7**:321-333.

Fukase, T., Matsuda, Y., Akihara, S. and Itagaki, H. (1985) Purification and some properties of cysteine protease of *Spirometra erinacei* plerocercoid (Cestoda; Diphyllobothriidae). *Jpn. J. Parasitol.*, **34**:351-360.

Ishii, A. (1973) Indirect fluorescent antibody test in human sparganosis. *Jpn. J. Parasitol.*, **22**:75-78.

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

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

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.

Ohnishi, Y., Takakura, Y., Yoshimura, H. and Tsubota, N. (1986) Antibody production in experimental sparganosis of rabbits: Agglutination and indirect fluorescent antibody techniques. *Jpn. J. Parasitol.*, **35**:25-33.

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. *Methods in Enzymol.*, **92**:377-391.

=국문초록=

스파르가눔 항원단백질에 대한 스파르가눔증 환자 혈청의 반응 양상

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스파르가눔증을 혈청학적으로 진단하기 위하여 효소면역측정법을 실시할 경우, 그 민감도와 특이도가 높다고 이미 보고되었다. 실제로 조직기생충 감염이 의심되는 환자에 대하여 특이 IgG항체가를 일상적으로 검사하면 중추신경계 환자중에서 뇌스파르가눔증은 드물지 않게 발견할 수 있다. 그러나 혈청학적 진단에 이용되는 스파르가눔 조항원(粗抗原)은 조충증(條蟲症), 유구낭미충증이나 포충증 환자의 혈청과 비특이적인 교차반응을 일으키는 경우가 있다. 이러한 교차반응의 문제를 해결하려면 우선 스파르가눔 항원의 구성 단백질이 환자혈청과 반응하는 양상을 알아야 할 필요가 있다. 이 연구에서는 외과적으로 스파르가눔증을 확인한 환자 15명에서 얻은 혈청이 스파가눔의 생리식염수 추출액의 항원단백질중 어느 분획과 반응하는지 검토하였다. 그리고 뇌유구낭미충증 환자 24명(그중 8명은 효소면역측정법으로 교차반응이 있었음)의 혈청과 교차반응을 일으키는 단백질 분획을 관찰하였다.

스파르가눔충체의 생리식염수 추출액을 10~15% linear gradient gel에서 SDS-폴리아크릴아마이드 전기영동을 실시하여 단백질대 30개를 구별할 수 있었다. 분리한 단백질대(帶)를 nitrocellulose 종이에 옮긴 후 스파르가눔증 환자 혈청 및 conjugate와 차례로 반응시키고 발색반응을 일으킨 결과(효소면역 전기영동이적법, immunoblot), 스파르가눔 항원단백질중 29kDa 및 36kDa가 가장 많이 또 가장 진하게 반응한 것이어서 민감하고 항원성이 높은 단백질 분획이라고 판단하였다. 그리고 위의 단백질 이외에도 158kDa, 130kDa, 107kDa, 78kDa, 72kDa, 52kDa, 23kDa, 21kDa, 15kDa 및 6kDa 등에도 반응이 있었다.

유구낭충증 환자 24명의 혈청중 스파르가눔 항원에 혈청학적 교차반응이 있었던 혈청은 36kDa 및 29kDa 단백질에 반응하고 있어 이 단백질이 스파르가눔의 종 특이(種特異)단백질 항원은 아닌 것으로 판단되나 그 성질에 대해서는 앞으로 더 추구할 가치가 있다고 생각한다.