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Partially purified *Toxoplasma gondii* antigens by immunoaffinity chromatography

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Abstract: Tachyzoite antigens of *Toxoplasma gondii* (RH) were partially purified by immunoaffinity chromatography. The cultivated *Toxoplasma in vivo* (mouse) and *in vitro* (Hep-2 cell) and peritoneal fluid of *T. gondii* infected mice were collected for antigen analysis. Tachyzoite antigens collected from infected mouse showed positive bands of 76 kDa, 70 kDa, 64 kDa, 53 kDa, 46 kDa, 44 kDa, 41 kDa, 35 kDa, 25 kDa, 18 kDa, and 13 kDa on immunoblot with anti-*Toxoplasma* rabbit sera, and those from infected Hep-2 cells revealed reactive bands of 70 kDa, 64 kDa, 53 kDa, 35 kDa, 28 kDa, and 13-10 kDa. After applying to an IgG-Sepharose column, two elution peaks, E-1 and E-2 fractions, were obtained from both soluble antigen of *T. gondii* and the peritoneal fluid of infected mice, respectively. Immunoblots of soluble antigen with immunized rabbit sera revealed positive bands of 97 kDa, 63 kDa, 53 kDa and 35 kDa from E-1 fraction and 53 kDa and 35 kDa from E-2. In the case of the eluted peaks from mice peritoneal fluid, E-1 showed protein bands of 84 kDa, 76 kDa, 53 kDa and 29 kDa bands and 53 kDa and 45 kDa from E-2 on immunoblots. Serum IgG antibody titer of mice immunized with *T. gondii* tachyzoites was increased on 1 week after booster immunization when analysed by ELISA using crude antigen, while it was elevated on 3 weeks after booster immunization by ELISA using purified antigen.

Key words: *Toxoplasma gondii* (RH), antigen purification, immunoaffinity chromatography, SDS-PAGE/immunoblot, ELISA

INTRODUCTION

Human infection with *Toxoplasma gondii*, an obligate intracellular protozoan parasite, produce a serious disease in congenitally infected infants and in immunocompromized persons such as AIDS patients or recipients of organ transplantation, although most of immunocompetent hosts are asymptomatic. The diagnosis of toxoplasmosis is based on serologic tests, isolation of *Toxoplasma* antigen, cultivation, histologic observation, CT and sonographic examination, but serologic tests are common method for the diagnosis. They include Sabin-Feldman dye test, indirect hemagglutination test (IHA), latex agglutination (LA), indirect fluorescent antibody test (IFA) and ELISA. Combination of serum IgG, IgM, and/or IgA antibody titers is helpful to distinguish between acute and chronic or between congenital and acquired infections.

Isolation of *Toxoplasma* antigens may be helpful for the development of new diagnostic method (Ho-Yen and Joss, 1992). The 30 kDa surface antigen of *T. gondii* tachyzoite is highly immunogenic and a useful candidate for diagnosis and other surface proteins of 43 kDa, 35

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kDa, 22 kDa, and 14 kDa are also known to be important antigens of *Toxoplasma* (Sharma *et al.*, 1983; Couvreur *et al.*, 1988). Excretory-secretory antigens of *Toxoplasma* located in dense granules, parasitophorus vacuoles and rhoptries play certain roles in host cell invasion and multiplication in the cells (Charif *et al.*, 1990). *Toxoplasma* antigens are shown to have some differences according to various factors such as strains, stages, acute or chronic infection, sera of human and experimental animals, individual immunity, *etc.* (Ware and Kasper, 1987).

This study was aimed to clarify antigens of *T. gondii* (RH) by immunoaffinity chromatography, and to observe the titers of serum antibody of infected mouse by ELISA.

MATERIALS AND METHODS

Preparation of soluble antigens of *T. gondii* and peritoneal fluids from infected mouse

Tachyzoites of *T. gondii* (RH strain), serially passaged in ICR mice every 3 to 4 days, were collected from peritoneal exudate of mice. Hep-2 cell cultured in MEM containing 10% fatal calf serum were infected with *T. gondii*, and tachyzoites were harvested after 3 to 4 days, and washed three times with PBS. They were centrifuged at 500 rpm for 3 min and at 2,000 rpm for 10 min, and stored at -20°C until used. Homogenate of *T. gondii* tachyzoites was centrifuged at 4°C, 6,000 rpm for 20 min after sonication at 100 watt (10 sec × 10 times) in an ice beaker. The supernatant was centrifuged again at 4°C, 10,198 rpm (10,000 *g*) for 1 hr and concentrated by ultrafiltration with Centriplus 10 (Amicon) for soluble antigen. The protein content was determined by the method of Lowry *et al.* (1951).

BALB/c mice were inoculated intraperitoneally with 10^3 tachyzoites of *T. gondii* (RH). After 7 days, 4 ml of PBS were injected into the peritoneal cavity of mice and then the exudate was collected for centrifugation. The supernatant was concentrated by ultrafiltration with Centriplus 10 and stored at -20°C until used. Peritoneal fluid of uninfected mouse was collected after PBS injection as a control.

Antiserum

Rabbits were injected intradermally with a mixture of 500 μl soluble antigen from 2.5×10^8 tachyzoites of *T. gondii* and 500 μl of Freund's complete adjuvant. Booster injection was performed after 4 weeks, and rabbits were bled on 20 days after booster immunization. BALB/c mice were injected intradermally with a mixture of 2.6×10^7 frozen tachyzoites and 100 μl of Freund's complete adjuvant. Booster injection was performed after 2 weeks, and mice were bled on 1, 2, 3 weeks after booster immunization. Immune sera were stored at -20°C and antibody titer was determined by ELISA.

Isolation of IgG from immunized rabbit sera

The absorbance for serum IgG level of immunized rabbit was 0.83 ± 0.02 by ELISA (control 0.10 ± 0.006). The immunoaffinity chromatography was performed by descriptions of Johnstone and Thorpe (1996).

Ammonium sulfate was added to 5 ml of serum pre-warmed to 25°C in order to make a 18% (w/v) solution. Mixtures were incubated for 30 min at 25°C and centrifuged at 3,000 *g* for 30 min at 25°C. After discarding the supernatant, protein precipitate was redissolved in distilled water to make a volume up to 2.5 ml. Ammonium sulfate was added to the solution to make a 14% (w/v) solution, and precipitate was redissolved in water up to 1.5 ml after a series of incubation and centrifugation. The solution was dialysed aganist PBS or 0.1 M phosphate (pH 8.0).

Protein A Sepharose 4B (Pharmacia, Sweden) was pre-swelled and packed in a column (0.8 × 10 cm, Bio-Rad, USA). Ammonium sulfate treated serum precipitate was applied to protein A Sepharose 4B column pre-equilibrated with 0.1 M phosphate (pH 8.0). After loading the serum, column was washed with equilibrating buffer to free of non-binding proteins. The bound IgG was eluted with 0.1 M glycine-HCl (pH 3.0) and each fraction of 0.5 ml was collected at a flow rate of 0.1 ml/min. Fractions were transferred immediately to milder pH condition with a few drops of 1 M Tris-HCl (pH 9.0) because the elution buffer of low PH is quite harsh.

Purification of *T. gondii* antigen

CNBr-activated Sepharose 4B was swelled in 1 mM HCl (ice cold) and coupled with immunoglobulins in coupling buffer (0.1 M $NaHCO_3$, 0.5 M NaCl, pH 8.3). The mixtures were rotated for overnight at 4°C and blocked by adding 1 M ethanolamine (pH 8.0) for 2 hrs at room temperature. They were alternately washed for four or five times with 0.1 M acetate buffer (pH 4.0) and coupling buffer each containing 0.5 M NaCl. Immunoglobulin coupled to CNBr-activated Sepharose 4B was packed to a column (0.8 × 10 cm, Bio-Rad, USA). Soluble antigen of *T. gondii* or peritoneal fluids of infected mice were applied to the column pre-equilibrated with 40 mM phosphate (pH 7.2). After challenging the antigens, the column was washed with equilibrating buffer until it became free of non-binding proteins. The bound antigens were eluted with 50 mM glycine-HCl (pH 2.5) containing 0.15 M NaCl and then 0.1 M carbonate (pH 11.0). Each fraction of 800 μl was collected at a flow rate of 0.1 ml/min. The fractions of high absorbance at 280 nm were pooled and concentrated using Minicon B 15 (cut-off; 15,000 Da, Amicon, USA).

SDS-PAGE/immunoblot

SDS-PAGE was carried out by the method of Laemmli (1970) using 12% separating gel and 4% stacking gel. Protein bands were separated through the constant current of 15 mA at 4°C and stained with Coomassie brilliant blue R 250 (Merck, Germany).

After electrophoresis, immunoblotting was performed by the method of Towbin *et al.* (1979) and Tsang *et al.* (1983). Protein bands on gels were transferred to nitrocellulose membrane (0.45 μm, Amersham, UK) for 3 hrs at 70 V, 4°C. The membranes were saturated for 1 hr at 37°C in blocking buffer (0.1 M PBS-0.2% Tween 20, 5% skim milk, pH 7.5). After washing with buffer (PBS-0.2% Tween 20, pH 7.5), a 1:500 dilution of anti-*T. gondii* rabbit serum was applied to the strips for 1 hr followed by incubation with peroxidase conjugated anti-rabbit IgG (Cappel, PA, USA), 1:1,000 dilution, for 1 hr at 37°C. The reaction was developed with the substrate, 50 mg diaminobenzidine (DAB, Bio-Rad, USA) and 10 μl of 30% H_2O_2 in 100 ml PBS for 10-20 min.

ELISA

ELISA was performed according to the modified method of Voller *et al.* (1976). The crude antigen (5 μg/ml) was coated in a 96 well plate. A 1:100 diluted infected mouse serum, 1:1,000 diluted peroxidase-conjugated anti-mouse IgG and orthophenylene diamine were used. ELISA with purified antigen was done as the same procedure except using 2 μg/ml of protein and 1:10 diluted infected mouse serum. Two wells were tested for one mouse serum. The optical density was read at 490 nm with ELISA reader. Statistical analysis was done with spss/Mann-Whithey U-test.

RESULTS

Antigenic fractions of *T. gondii*

To investigate the difference of antigenic fractions of *T. gondii* according to culture method, tachyzoites were cultured in the peritoneum of ICR mice and in Hep-2 cell, *in vitro*. On SDS-PAGE, the major bands of *T. gondii* tachyzoites from ICR mice were 112 kDa, 76 kDa, 70 kDa, 53 kDa, 46 kDa, 44 kDa, 41 kDa, 35 kDa, 25 kDa, 22 kDa, 18 kDa, 15 kDa, and 13 kDa, and those of tachyzoites cultured in Hep-2 cell were 100 kDa, 70 kDa, 68 kDa, 58 kDa, 53 kDa, 46 kDa, 37 kDa, 35 kDa, 28 kDa, 27 kDa, 18 kDa, 15 kDa, and 13 kDa.

When transferred to nitrocellulose membrane, tachyzoites recovered from peritoneal fluid of ICR mice showed positive bands of 76 kDa, 70 kDa, 64 kDa, 53 kDa, 46 kDa, 44 kDa, 41 kDa, 35 kDa, 25 kDa, 18 kDa, and 13 kDa, while bands of 70 kDa, 64 kDa, 53 kDa, 35 kDa, 28 kDa and 13-10 kDa were reacted with anti-*Toxoplasma* rabbit sera in those of cultured in Hep-2 cell (Fig. 1).

Soluble antigens of *T. gondii* were applied to an IgG-Sepharose column. Two elution peaks were obtained with 50 mM glycine-HCl (pH 2.5) containing 0.15 M NaCl as E-1, and 0.1M carbonate (pH 11.0) as E-2. SDS-PAGE analysis revealed four protein bands of 63 kDa, 53 kDa, 45 kDa, and 35 kDa from E-1 fraction, and two bands, 53 kDa and 35 kDa, from E-2 fraction. Immunoblot with immunized rabbit

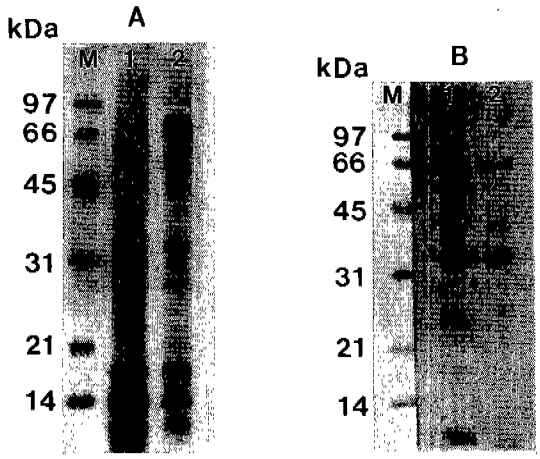

Fig. 1. SDS-PAGE (A) and immunoblot (B) analyses of *T. gondii* tachyzoite. M, marker; lane 1, *T. gondii* tachyzoite from infected mouse peritoneum; lane 2, *T. gondii* tachyzoite cultured *in vitro* with Hep-2 cell.

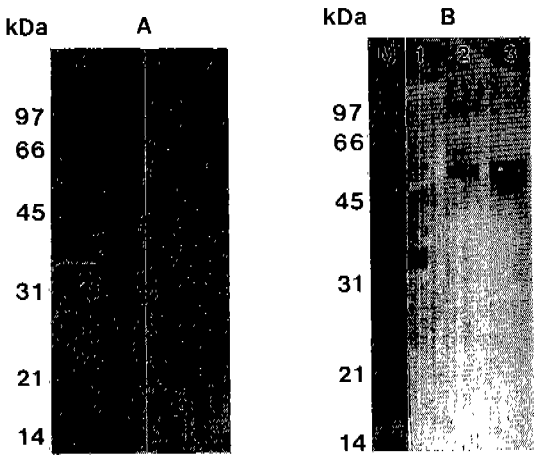

Fig. 2. SDS-PAGE (A) and immunoblot (B) analyses of crude or purified *T. gondii* antigen. M, marker; lane 1, *T. gondii* tachyzoite lysate; lane 2, the first elution peak of purified *Toxoplasma* antigen; lane 3, the second elution peak of purified *Toxoplasma* antigen.

sera revealed four reactive bands, 97 kDa, 63 kDa, 53 kDa and 35 kDa from E-1 fraction, and two bands, 53 kDa and 35 kDa, from E-2 fraction (Fig. 2).

Antigens from peritoneal fluid of infected mouse

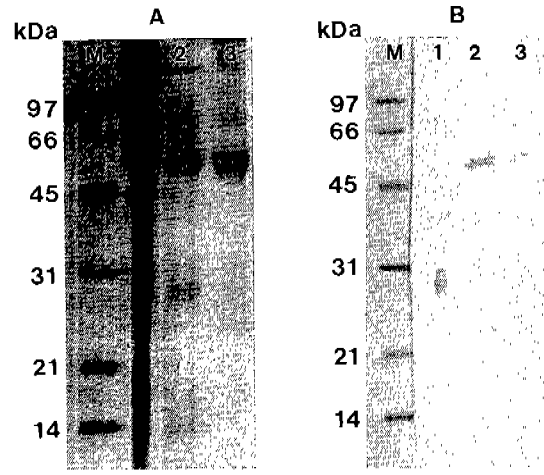

Fig. 3. SDS-PAGE (A) and immunoblot (B) analyses of *Toxoplasma* infected mouse peritoneal fluid and purified antigen. M, marker; lane 1, ascitic fluid from infected mouse; lane 2, the first elution peak of purified antigen; lane 3, the second elution peak of purified antigen.

Peritoneal fluid of infected mouse showed more than 21 bands ranging from 160 kDa to 13 kDa, while that of normal mouse had more than 13 bands from 183 kDa to 27 kDa by SDS-PAGE analysis. Immunoblot demonstrated antigenic bands with the molecular weight of 76 kDa, 53 kDa, 35 kDa and 29-28 kDa from peritoneal fluid of infected mouse. Normal mouse peritoneal fluid showed 76 kDa, 53 kDa and 29-28 kDa bands, however, their reaction was weaker than those of infected mouse.

When infected mouse peritoneal fluid was applied to an IgG-Sepharose column, two major fractions, E-1 and E-2, were eluted, too. Antigenic bands of 106 kDa, 97 kDa, 76 kDa, 53 kDa and 29 kDa from E-1, and 76 kDa and 53 kDa from E-2 fractions were identified on SDS-PAGE. Protein bands of 84 kDa, 76 kDa, 53 kDa and 29 kDa from E-1, and 53 kDa and 45 kDa from E-2 fraction were reactive with immunized rabbit sera (Fig. 3).

IgG antibody titers of immunized mouse sera

Serum IgG antibody titer of mouse immunized with *T. gondii* tachyzoite was increased to 0.42 ± 0.149 or 0.59 ± 0.01 (control 0.25 ± 0.03) on 1 week after booster immunization by

Table 1. Serum IgG antibody levels of *T. gondii* immunized mice by ELISA using crude antigen

	Weeks after immunization	No. of mouse	Serum IgG Ab titer (Mean ± 2 SD)
E-1)	1 week after the 1st Imm	4	0.2 ± 0.046
	1 week after the 2nd Imm	4	0.42 ± 0.149
	2 weeks after the 2nd Imm	3	0.42 ± 0.061[a]
E-2)	1 week after the 2nd Imm	5	0.59 ± 0.01[a]
	3 weeks after the 2nd Imm	4	0.64 ± 0.08[a]
E-3)	2 weeks after the 2nd Imm	5	0.75 ± 0.004[a]
	3 weeks after the 2nd Imm	5	0.75 ± 0.004[a]
	5 weeks after the 2nd Imm	6	0.75 ± 0[a]
	Normal mouse serum	4	0.25 ± 0.03

[a] $p<0.05$

Table 2. Serum IgG antibdoy levels of *T. gondii* immunized mice by ELISA using purified antigen

	Weeks after immunization	No. of mouse	Serum IgG Ab titer (Mean ± 2 SD)
E-1)	1 week after 1st Imm	3	0.26 ± 0.051
	1 week after 2nd Imm	3	0.36 ± 0.14
	2 weeks after 2nd Imm	3	0.33 ± 0.098
E-2)	1 week after 2nd Imm	3	0.33 ± 0.039
	3 weeks after 2nd Imm	3 (PA)[b]	0.47 ± 0.067[a]
		3 (CA)[b]	0.58 ± 0.025[a]
	Normal mouse serum	2 (PA)	0.31 ± 0.024
		2 (CA)	0.18 ± 0.005

[a] $p<0.05$, [b] PA, purified antigen of *T. gondii*; CA, crude antigen of *T. gondii*.

ELISA with crude antigen (Table 1). And it was elevated to 0.47 ± 0.067 (control 0.31 ± 0.024) on 3 weeks after booster immunization in case of using purified *Toxoplasma* antigen (Table 2).

DISCUSSION

The antigens of *Toxoplasma* are complex and diverse. Three infective stages of *Toxoplasma*, tachyzoite, bradyzoite and oocyst (sporozoite) have specific antigens as well as common antigens. The antigens of 54 kDa to 63 kDa and 26 kDa to 29 kDa were demonstrated for both stages, tachyzoite and bradyzoites of *T. gondii* (Makioka *et al.*, 1991). *T. gondii* sporozoites have two major membrane proteins of 67 kDa and 25 kDa, which are not present in tachyzoite, and deficient of major tachyzoite protein, p30 (Kasper *et al.*, 1984).

In the present study, *Toxoplasma* lysate and infected mouse peritoneal fluid were applied to affinity chromatography for partial purification of *Toxoplasma* antigens. Tachyzoites of *Toxoplasma* (RH) collected from infected mouse peritoneum and Hep-2 cell infection, *in vitro* and supernatant of infected mouse peritoneal fluid were analysed for antigenic identification and these three soluble *T. gondii* antigen preparations were compared. The crude antigens from mouse subcultured and Hep-2 cell cultivated parasites revealed same molecular weights of 70 kDa, 64 kDa, 53 kDa, 35 kDa, and 13 kDa. In partially purified *Toxoplasma* antigen using affinity chromatography, two elution peaks, E-1 fraction showing 63 kDa, 53 kDa, 45 kDa, and 35 kDa, and E-2 of 53

kDa and 35 kDa, were obtained. The 53 kDa and 35 kDa were revealed in *Toxoplasma* crude antigen and partially purified antigens of E-1, E-2 and the 63 kDa was found in crude antigen and purified antigen, E-1. The 97 kDa antigen that was observed in the purified antigen, E-1, but not in the *Toxoplasma* crude antigens may be a concentrated antigenic protein or a false positive reaction on immunoblotting. We lost several surface proteins of *T. gondii* during the course of protein preparation and unfortunately, a major surface antigen of 30 kDa (p30), was not detected in this experiment. But we found p30 in the protein bands of crude antigen of *T. gondii* and supernatants of infected mouse peritoneal fluid by SDS-PAGE. In our experiment, antigenic differences between *in vivo* and *in vitro* cultivated *Toxoplasma* tachyzoites were not distinct. But more antigenic bands were observed from *in vivo* cultivated tachyzoites by immunoblotting.

The peritoneal fluid of infected and uninfected mice contained numerous proteins but were able to assess antigenic proteins with immune rabbit sera. The supernatant of infected mouse peritoneal fluid contains antigens referring to as lytic and excretory-secretory antigens of *Toxoplasma*. Immunoblotting analysis of supernatants of *Toxoplasma* infected and uninfected mice peritoneal fluids revealed the same antigen bands of 76 kDa, 53 kDa, 29-28 kDa except 35 kDa with different intensity of reaction. Three bands of 76 kDa, 53 kDa and 29-28 kDa showed cross reactions with normal mouse peritoneal fluid but their antigenicity could not be ruled out. In purified infected mouse peritoneal fluid, two elution peaks were exhibited as E-1 containing 84 kDa, 76 kDa, 53 kDa, and 29 kDa, and E-2 containing 53 kDa and 45 kDa. The comparison of the antigenic profiles shown by the lytic antigens and infected mouse peritoneal fluid preparations seemed to indicate somatic antigens of *Toxoplasma* because both antigenic preparations were similar on immunoaffinity chromatography. It may be possible that somatic and excretory-secretory antigens of *Toxoplasma* have common bands. In our results, major antigens in *Toxoplasma* extract detected by immunoaffinity chromatography were 76 kDa, 63 kDa, 53 kDa, 35 kDa, and 29 kDa.

Choi *et al.* (1988) found some membrane proteins of RH strain tachyzoite, 30 kDa and 52 kDa glycoproteins with specific antigenicity. *Toxoplasma* tachyzoites contain a potent NTPase (nucleoside triphosphate hydrolase) that has a molecular weight of 63 kDa and is localized in dense granules. During the invasion of the host cell and formation of the parasitophorous vacuoles, NTPase is rapidly released by exocytosis into the vacuolar lumen. NTPase in the tachyzoite of *T. gondii* is detected as a circulatory antigen in infected mice sera (Asai *et al.*, 1987; Sibley *et al.*, 1994). In both *T. gondii* antigen preparation from the lytic extract and culture supernatants were revealed same molecular weight components of 57 kDa, 52 kDa, 43 kDa, 38 kDa, 35 kDa, 30 kDa, and 20 kDa. Somatic antigens were also present in *T. gondii* culture supernatant (Bessieres *et al.*, 1992). In previous report, we found the molecular weight of 30 kDa and 20 kDa in culture supernatant and 33 (p30) kDa and 45 kDa from infected mouse peritoneal fluid as excretory-secretory antigens of *T. gondii*, RH strain (Ahn *et al.*, 1994).

Toxoplasmosis is mostly diagnosed by the serologic test using specific antibodies, IgG, IgM and IgA. Im *et al.* (1991) examined a total of 618 sera from the pregnant women for prenatal care by IFA and ELISA. The positive results of 6.6% by IFA and 7.0% by ELISA of tested pregnant women sera were observed without agreement of two tests. In human sera, the positive titer by ELISA was about two times higher than by ILA (Choi *et al.*, 1992). Among 899 sera of pregnant women, 4.3% of tested sera showed positive reaction by ELISA and 0.8% by LAT (Ryu *et al.*, 1996). It may be a different antigenic epitope recognized by latex agglutination and ELISA (Makioka *et al.*, 1991). Recently, the recombinant *T. gondii* polypeptides with molecular weight of 25 kDa, and 41 kDa were used as antigen of ELISA for diagnosis of human toxoplasmosis, and the ELISA with recombinant antigen showed a higher sensitivity to acute toxoplasmosis (Tenter and Johnson, 1991). And Wee *et al.* (1992) reported IgM capture ELISA using monoclonal antibody that detected as 22 kDa, 41

kDa membrane antigen and the results of this assay was specific and favorable compared with commercial kits. The 30 kDa of *Toxoplasma* antigen using monoclonal antibody was recognized to be more specific than crude antigen in ELISA test (Shin *et al.*, 1997). In recent human toxoplasmosis, the 35 kDa band was noted in the earliest sera of patient and other bands of 39-40 kDa, 43 kDa, and 48 kDa were revealed. Three month after, 29 kDa, 35 kDa, 39-40 kDa, 53-54 kDa, and 59-60 kDa bands with high intensities were followed (Verhofstede *et al.*, 1988).

Few serologic tests with high sensitivity and specificity for toxoplasmosis are available and, therefore, combination of more than two kinds of test is needed for accurate diagnosis. Another aim of the present study was to compare the applicability as an antigen for ELISA between the crude and purified antigens of *Toxoplasma*. In our experiment, the crude antigen of *Toxoplasma* could be more easily detected by immunized mouse sera. The increased antibody titer was observed in mouse serum on 1 week after the 2nd immunization by ELISA using crude antigen. And we observed elevated antibody titer on 3 weeks after booster immunization by ELISA with purified antigen. And purified antigen of *Toxoplasma* is not always better than crude antigen in the serologic test. In ELISA test both antigens were useful for preliminary test of *Toxoplasma* infection.

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= 초록 =

Immunoaffinity chromatography를 이용한 톡소포자충 항원의 부분정제

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톡소포자충, RH주 tachyzoite의 항원성 단백질에 대하여 알아보고자 마우스 복강 내 또는 *in vitro*에서 Hep-2 cell에 계대한 톡소포자충과 감염마우스의 복강액으로 SDS-PAGE/immunoblot을 시행하였다. 또 immunoaffinity chromatography를 이용하여 톡소포자충 항원을 정제한 후 SDS-PAGE/immunoblot을 시행하여 항원을 분리하였으며, 톡소포자충 조항원 및 정제항원으로 면역시킨 마우스 혈청을 이용하여 IgG-ELISA를 시행하였다. 톡소포자충 용해물을 면역시킨 토끼의 항혈청으로 immunoblot을 시행하였을 때 마우스 복강 내로 계대한 톡소포자충에서 76 kDa, 70 kDa, 64 kDa, 53 kDa, 46 kDa, 44 kDa, 35 kDa, 25 kDa, 18 kDa 및 13 kDa의 항원대가 관찰되었으며, *in vitro*에서 Hep-2 cell에 배양한 톡소포자충은 70 kDa, 64 kDa, 53 kDa, 35 kDa, 25 kDa 및 13-10 kDa의 항원대가 관찰되어 마우스 복강 내에 계대한 톡소포자충에서 더 많은 항원대가 관찰되었다. 마우스 복강 계대한 tachyzoite 용해물을 immunoaffinity를 시행하여 부분 정제한 후 면역시킨 토끼의 항혈청으로 immunoblot을 하였을때 E-1에서는 97 kDa, 63 kDa, 53 kDa 및 35 kDa이, E-2에서는 53 kDa 및 35 kDa이 나타났다. 한편 감염 마우스 복강액에서는 76 kDa, 53 kDa, 35 kDa 및 29-28 kDa의 항원대가 관찰되었으며, 정상 마우스 복강액에서도 약하게 76 kDa, 53 kDa 및 29-28 kDa의 반응대가 관찰되었다. 감염 마우스 복강액을 IgG-Sepharose column을 통과시킨 후 시행한 immunoblot에서 E-1은 84 kDa, 76 kDa, 53 kDa 및 29 kDa이, E-2에서 53 kDa 및 45 kDa의 항원대가 관찰되었다. 이 실험에서 immunoaffinity chromatography를 이용하여 톡소포자충 용해물 및 분비물에서 76 kDa, 63 kDa, 53 kDa, 35 kDa 및 29 kDa의 항원을 분리하였다. 톡소포자충의 조항원과 정제항원 (감염 마우스 복강액, E-1)으로 IgG-ELISA를 시행한 결과, 조항원의 경우 2차 면역 후 1주 후부터 IgG 항체가 증가하였고, 정제 항원은 2차 면역 후 3주 후부터 IgG 항체가 증가하였다 ($p<0.05$).

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