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Use of a Purified Major Surface Protein of *Toxoplasma gondii* in a Latex Agglutination Test

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Abstract

A major surface protein (P30) of *Toxoplasma gondii* was tested for possible use in a latex agglutination test for serodiagnosis of toxoplasmosis. We purified P30 by affinity chromatography with monoclonal anti-P30 antibody, sensitized latex particles with it and performed the agglutination test by comparing it with dye test (DT) and a commercial kit of LA (LA(MT); Eiken Chemical Co., Tokyo). The frequencies of respective titers of LA with P30 (LA(P30)) on 48 sera revealed a bimodal distribution curve, thereby titers above 1:16 were regarded as positive. Qualitative agreement between LA(P30) and DT attained to 93.8% which appeared to be the same as that between LA(MT) and DT. The titers of LA(P30) obtained by testing sera from a general human population were slightly lower than DT titers. In acute rabbit toxoplasmosis, the titers of LA(P30) elevated in parallel with those of LA(MT) not with those of DT following infection.

From these results, it was considered that the membrane antigens other than P30 would also be needed for the LA to be a more useful candidate for DT.

Key words: serodiagnosis, toxoplasmosis, latex agglutination test, purified protein

Introduction

Several laboratories have produced monoclonal antibodies (mAb) against the surface of tachyzoites of *Toxoplasma gondii* (Handman *et al.*, 1980; Johnson *et al.*, 1981; Kasper *et al.*, 1983; Rodriguez *et al.*, 1985). Most of these antibodies recognized a major radioiodinated surface protein with an apparent molecular weight of 27,000 to 30,000, as measured by sodium dodecyl sulfate polyacrylamide gel electrophoresis, which has been named P30 (Kasper *et al.*, 1983). Santoro *et al.* (1985) first used purified P30 for serodiagnosis in an enzyme-linked immunosorbent assay (ELISA) and demonstrated that all patients with both acute and chronic toxoplasmosis presented significant high levels of anti-P30 antibodies. They also used mAb to P30 for detection of IgM to *T. gondii* in ELISA (Cesbron *et al.*, 1985).

Surface membrane antigens are considered to be responsible for dye test (DT), which is regarded as a most reliable method for serodiagnosis of toxoplasmosis. We tried to generate mAb to P30. One mAb generated was highly positive in DT and directed to the same protein as P30 named by Kasper *et al.* (1983). Also we characterized it as an important antigen for DT (Makioka *et al.*, 1989). ELISA is a useful tool for serodiagnosis but is laborious and time-consuming, and also needs special instrument. In this respect, the LA may be simpler and easier to perform than ELISA. The present study was aimed to examine whether application of P30 in the LA was useful for serodiagnosis of toxoplasmosis by comparing it with DT and a commercial kit of LA (Eiken Chemical Co., Tokyo).

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Materials and Methods

Monoclonal antibody

Hybridoma secreting monoclonal antibody (mAb) to P30 has been described elsewhere (Makioka *et al.*, 1989). Ascitic fluids were pro-

duced in pristane-primed BALB/c mice and the mAb (IgM) was purified by a 50% ammonium sulphate precipitation followed by a gel filtration on Sephadex G-200.

Toxoplasma *membrane antigens*

Parasites were harvested from the peritoneal exudate of mice infected with the RH strain of *T. gondii.* Debris and host cells were removed by filtration through a polycarbonate membrane (pore size, 3 μm; Nuclepore Co., Pleasanton, CA). The tachyzoites were washed three times with saline by centrifugation at 900 $\times$ *g* for 5 min and disrupted by treatment with distilled water and sonication. They were centrifuged at 40,000 $\times$ *g* for 30 min to separate membranes from cytoplasmic soluble antigens. The membrane pellets were thoroughly washed with saline by centrifugation. To solubilize the membrane antigens, 5 volumes of 0.5% Triton X-100 were added to the pellet and allowed to agitate at 4°C overnight. The resulting suspension was centrifuged at 100,000 $\times$ *g* for 30 min and the supernatant was collected and used as the membrane antigens for isolation of P30.

Isolation of P30 by affinity chromatography

A pool of purified mAb anti-P30 was coupled to glutardialdehyde activated affinity adsorbent (Boehringer, Mannheim, West Germany) according to the manufacturer's recommended method. Briefly, 1 g of affinity adsorbent was suspended in 5 ml of 0.9% NaCl containing 20 mg/ml of mAb. After shaking for 4 hr at room temperature, the carrier was transferred to a column and washed with 1.5% NaCl until the eluate was free of protein (A_{280} < 0.005). Remaining free aldehyde groups on the carrier were saturated with 0.3 M ethanolamine-HCl, pH 7.5 for 1 hr. After washing sequentially with 50 ml 0.9% NaCl, 50 ml 0.5 M glycine, pH 3.5, and 100 ml 0.9% NaCl, the immunoadsorbent was stored at 4°C. Isolation of P30 was carried out with the adsorbent. The membrane antigens were applied to the column and then washed sufficiently with 3% NaCl. The adsorbed antigen was eluted with 3 M NaSCN. After dialysis against phosphate buffered saline, pH 7.2 (PBS), the eluted proteins were stored at —80°C.

SDS polyacrylamide gel electrophoresis (SDS-PAGE)

Antigen preparation was dissolved in SDS sample buffer and boiled for 2 min. SDS polyacrylamide gel electrophoresis was performed using a Tris-glycine buffer, pH 8.3, containing 0.1% SDS as described by Laemmli (1970) on 15% slab gels. After electrophoresis gels were stained with Coomassie blue.

Protein content was measured by the method of Lowry *et al.* (1951), using bovine serum albumin as a standard.

Sensitization of latex particles with P30

Latex particles were coated with P30 by the method of Tsubota and Ozawa (1977).

Human sera

Forty eight sera tested were chosen at random from general outpatients at Jikei University hospital in Tokyo.

*Rabbit anti-*T. gondii *sera*

Rabbits were infected intraperitoneally (i.p.) with 5,000 bradyzoites of the avirulent Fukaya strain of *T. gondii.* Rabbit antisera were obtained by bleeding just before infection and at various times thereafter.

Performance of serologic tests

The latex agglutination test with P30, designated LA(P30), was performed in a similar way for the commercial kit of LA (Toxo-MT: Eiken Chemical Co., Tokyo), designated LA(MT), as previously described (Kobayashi *et al.*, 1977). The DT was performed by a modification (Kobayashi *et al.*, 1968) of the technique described by Frenkel and Jacobs (1958).

Results

Purification and sensitization of protein P30

We used the affinity adsorbent, activated by glutardialdehyde, for isolation of P30 as it was reported that this adsorbent was better than

CNBr-activated Sepharose to purify P30 (Santoro *et al.*, 1985). We also used *Toxoplasma* membrane antigens instead of whole extract to apply the column. Our preparation of P30 was analysed by SDS-PAGE and showed a single band of 30,000 daltons although a faint band of 32,000 was also observed (Fig. 1). We determined optimal concentration of P30 for sensitizing latex particles and found that 60-70 μg of P30/mg latex particles was optimal. In the LA kit, 10—20 μg of toxoplasma protein/mg latex particles was used for sensitization.

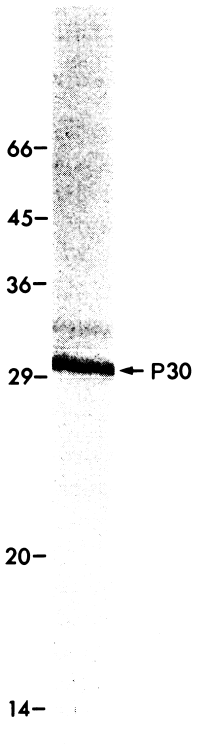

Fig. 1. Purified protein P30 after resolution by polyacrylamide gel electrophoresis in the presence of SDS. Numbers represent molecular weight standards: bovine serum albumin (66,000), egg albumin (45,000), glyceraldehyde dehydrogenase (36,000), carbonic anhydrase (29,000), trypsin inhibitor (20,100), lactoalbumin (14,200).

Frequency distributions of LA(P30), LA(MT) and DT titers

We first examined frequencies of respective titers of the LA(P30) on 48 sera to determine critical titer for positivity. They revealed a bimodal distribution curve, thereby titers above 1:16 were regarded as positive (Fig. 2). The frequency distribution curves of LA(MT) and DT titers were also bimodal and titers above 1:32 and 1:16 were regarded as positive, respectively (Fig. 2). The criteria for DT and LA(MT) were consistent with the results previously described (Kobayashi *et al.*, 1977).

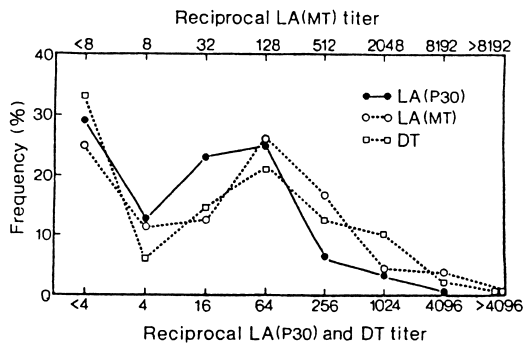

Fig. 2. Frequency distribution curves of LA(P30), LA(MT) and DT titers in 48 sera.

Table 1 Qualitative agreement between LA(P30) and DT with 48 sera

Agreement			Disagreement			
DT+ LA+ (%)	DT− LA− (%)	Total (%)	DT+ LA− (%)	DT− LA+ (%)	Total (%)	Grand total (%)
27 (56.3)	18 (37.5)	45 (93.8)	2 (4.2)	1 (2.1)	3 (6.2)	48 (100)

Titers above 1:16 in both tests are regarded as positive.

Qualitative agreements of LA(P30), LA(MT) and DT

The qualitative correlation between negative and positive results in the LA(P30) and DT on 48 sera is shown in Table 1. Both tests agreed in 93.8% of the sera. In the remaining sera (6.2%), two sera showed that the DT was positive and the LA(P30) was negative, and one serum which was negative in DT and positive in LA(P30). The qualitative correlation between LA(MT) and DT is shown in Table 2. Both tests agreed in 93.8% of the sera, which was almost similar to the previous results (Kobayashi *et al.*, 1977).

Quantitative correlations of LA(P30), LA(MT) and DT

Quantitative studies were carried out on 48 sera and titers compared in the LA(P30), LA(MT) and DT. The results are shown in Table 3 for the LA(P30) and DT and Table 4 for the LA(MT) and DT. The LA(P30) titers were most often equal or low (about a half) when compared with those of DT. The LA(MT) titers were twice as high as DT titers as previously described (Kobayashi *et al.*, 1977).

LA(P30), LA(MT) and DT titers in rabbit sera during acute Toxoplasma *infection*

Rabbits were infected i.p. with 5,000 bradyzoites of the avirulent Fukaya strain of *T.gondii* and bled at various intervals after infection. The results are shown in Fig. 3. The LA(P30) titer was low (1:12) at day 10 and thereafter increased to 1:512 at day 30 and 1:4096 at day 52. The LA(MT) titer was low (1:8) at day 7 and then elevated in parallel with LA(P30) titer and reached 1:4096 at day 35. On the other hand, the DT titer was $<1:4$ at day 7 and then showed a sharp rise from 1:32 at day 10 to 1:4096 at day 14, thereafter the level being kept up to 60 days postinfection.

Table 2 Qualitative agreement between LA(MT) and DT with 48 sera

Agreement			Disagreement			
DT+ LA+ (%)	DT− LA− (%)	Total (%)	DT+ LA− (%)	DT− LA+ (%)	Total (%)	Grand total (%)
28 (58.3)	17 (35.4)	45 (93.8)	1 (2.1)	2 (4.2)	3 (6.2)	48 (100)

Titers above 1:16 and 1:32 in DT and LA(MT) are regarded as positive, respectively.

Table 3 Anti-toxoplasma antibody titers observed by DT and LA(P30) in 48 sera

Reciprocal of DT titers	Reciprocal of LA(P30) titers													% Posi. by LA
	<2	2	4	8	16	32	64	128	256	512	1024	2048	Total	
<4	3	11	2										16	(0)
4			2		1								3	(33.3)
16				1	4	1	1						7	(85.7)
64				1	1	4	4						10	(90)
256							3	3					6	(100)
1024								1	1	2	1		5	(100)
4096												1	1	(100)
Total	3	11	4	2	6	5	8	4	1	2	1	1	48	(58.3)
(% Posi. by DT)	(0)	(0)	(0)	(100)	(83.3)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(60.4)	

Titers above 1:16 in both tests are regarded as positive.

Table 4 Anti-toxoplasma antibody titers observed by DT and LA(MT) in 48 sera

Reciprocal of DT titers	Reciprocal of LA(MT) titers																% Posi. by LA
	<2	2	4	8	16	32	64	128	256	512	1024	2048	4096	8192	16384	32768	Total	
<4	0	5	7	4													16	(0)
4					1		2										3	(66.7)
16					1	1	1	2	1	1							7	(85.7)
64							2	1	3	1	3						10	(100)
256								2	2		2						6	(100)
1024									1		1	2		1			5	(100)
4096																1	1	(100)
Total	0	5	7	4	2	1	5	5	7	2	6	2		1		1	48	(62.5)
(% Posi. by DT)	(0)		(0)	(0)	(50)	(100)	(60)	(100)	(100)	(100)	(100)	(100)		(100)		(100)	(60.4)	

Titers above 1:16 and 1:32 in DT and LA(MT) are regarded as positive, respectively.

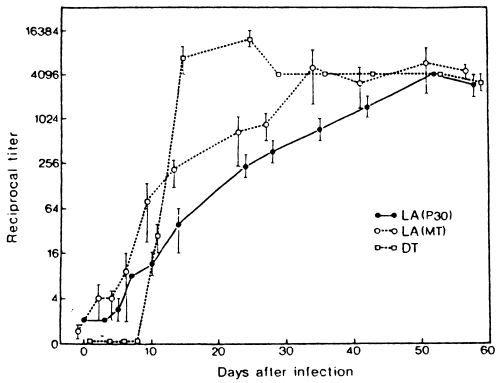

Fig. 3. LA(P30), LA(MT) and DT titers in rabbits infected with the avirulent Fukaya strain of *T. gondii*. Each point represents the mean ± standard error of those test titers from five rabbits.

Discussion

Preliminary studies have demonstrated the presence of anti-P30 antibodies (IgG and IgM) in sera of patients with *Toxoplasma* infection (Handman *et al.*, 1980; Johnson *et al.*, 1981; Kasper *et al.*, 1983). More recently, using affinity-purified P30, Santoro *et al.* (1985) demonstrated that all patients with either acute or chronic toxoplasmosis had significantly high levels of anti-P30 antibodies as assayed by ELISA. The latex agglutination test (LA) may be simpler and easier to perform than ELISA. The present study was aimed to evaluate the LA with P30 by comparing it with DT and the commercial kit of LA. As there are limitations in obtaining large amounts of P30 for sensitizing latex particles, the total number of sera we tested was few but frequency distribution curves of respective titers of the LA kit and DT and also a qualitative agreement between the two in the present study were almost the same as those of our previous study in which 412 sera were tested (Kobayashi *et al.*, 1977). The LA kit is most commonly used as a substitute for DT in Japan. We have already evaluated the kit and reported that it was specific and sensitive to detect the toxoplasma antibody and would provide a useful tool for screening of toxoplasmosis (Kobayashi *et al.*, 1977). However, the kit of LA is still inferior in specificity to DT. Antigens used for the LA kit are mainly cytoplasmic, soluble ones and are considered to contain few membrane antigens in which P30 is involved. Therefore, we expected higher qualitative agreement between LA(P30) and DT than that between LA(MT) and DT, but both agreement rates were almost the same. When compared with LA(MT), there was observed a tendency of decrease in number of false-positive sera but also that of increase in number of false-negative sera in the LA(P30). The latter may be related to the sensitivity of LA(P30). The results demonstrated that titers of LA(P30) were often lower than DT

titers. Similar results were also observed in rabbit antisera during acute *Toxoplasma* infection. This may be accounted for by the use of only a single antigen of P30. Handman *et al.* (1980) demonstrated that at least four major antigens containing P30 were immunoprecipitated from radioiodinated surface antigens by antisera to *T. gondii*. These membrane antigens other than P30 would also participate in DT.

From these results, it was considered that the membrane antigens other than P30 would also be needed for the LA to be a more useful candidate for DT. This possibility is now under investigation.

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