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Detection of Circulating Antigens by Latex Particles Coated with Anti-*Toxoplasma* Antibodies During Acute Infections with *Toxoplasma gondii* in Mice

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Introduction

In the serodiagnosis of acute toxoplasmosis, a titration of anti-*Toxoplasma* antibodies by Sabin-Feldman dye test and anti-*Toxoplasma* IgM antibodies is important and informative (Welch *et al.*, 1980 ; Kobayashi *et al.*, 1983). However, serological methods may be inconclusive or unreliable in some instances ; patients with underlying diseases causing suppressed antibody responses, patients receiving immunosuppressive therapy (Ruskin and Remington, 1976 ; Krick and Remington ; 1978), and certain cases of congenital toxoplasmosis (Stango, 1980). In those cases, a direct detection of antigenic components of *Toxoplasma gondii* in sera or body fluids might offer a valuable aid for rapid and specific diagnosis of an acute toxoplasmosis.

Recently, it was reported that circulating *Toxoplasma* antigens (c-Ag) are detectable by enzyme-linked immunosorbent assay (ELISA) (Raizman and Neva, 1975 ; van Knapen and Panggabean, 1977 ; Araujo and Remington, 1980) and radio immunoassay (RIA) (Kamei and Yoshiike, 1981) in the sera from patients with acute toxoplasmosis. However, ELISA and RIA are technically complicated, and special instruments are needed to read the results of reactions. Therefore, in the present study, we tried to develop a simple method for quantitative assay of c-Ag, and found that an application of agglutination test using latex particles coated with anti-*Toxoplasma* antibodies (Ag-LA) was satisfactory for this purpose.

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Materials and Methods

Mice. Outbred female ddY and inbred female C57BL/6 mice were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu). Five to ten mice were used in each experimental group.

Toxoplasma infection. Tachyzoites of the RH strain of *T. gondii* were obtained from the peritoneal cavity of mice which had been inoculated with the organisms 3 days earlier. The tachyzoites were suspended in phosphate-buffered saline (PBS), and washed by centrifugation at $700 \times g$ for 5 min three times. The sedimented organisms were resuspended in PBS at various concentrations. Mice were inoculated with 0.2 ml of the suspension intraperitoneally.

For a chronic *Toxoplasma* infection, mice were inoculated with 5×10^3 bradyzoites of the avirulent Fukaya strain of *T. gondii* intraperitoneally. Bradyzoites were obtained from the brains of the infected mice by treatment with 0.25% trypsin for 10 min as described previously (Suzuki *et al.*, 1981).

Ag-LA test. Anti-*Toxoplasma* IgG antibodies were obtained from the infected rabbit serum by passing through a Protein A-

Sepharose column. Latex particles were coated with the purified anti-*Toxoplasma* rabbit IgG by the method described by Tsubota and Ozawa (1977). A quantitative assay for c-Ag was performed by a microagglutination test using the antibody coated latex particles. An equal volume (0.025 ml) of latex particle suspension was added to each of doubling dilutions of sera made in microtiter trays, and the pattern of agglutination was read after the trays had stood at room temperature overnight.

Titration of anti-Toxoplasma antibodies. An indirect latex agglutination (LA) test using a commercial kit product (Eiken Chemical Co. Ltd., Tokyo) was performed for the titration of anti-*Toxoplasma* antibodies. For the titration of 2-mercaptoethanol (2-ME)-resistant anti-*Toxoplasma* antibodies, sera were incubated with equal volumes of 0.1 M 2-ME in PBS at 37°C for 1 hr before addition of the latex particles.

Preparation of Toxoplasma lysate antigens. Cellfree tachyzoites of the virulent RH strain were suspended in distilled water and lysed osmotically. Clear supernatant containing soluble *Toxoplasma* antigens were obtained by high speed centrifugation (10,000×*g* for 30 min). Protein concentration of the lysate was measured by the methods described by Lowry *et al.* (1951).

Results

Sensitivity of Ag-LA test. A sensitivity of Ag-LA test was examined using *Toxoplasma* lysate antigens. Two fold dilutions of the lysate antigens were made in a microtiter tray, and a equal volume of the antibody-coated particle suspension was added to the dilutions. An agglutination of the latex particles was observed in the antigens at protein concentrations of 1.25 μg/ml or more.

Reaction pattern of Ag-LA test. Agglutination patterns were examined in normal and infected mouse sera, which were obtained from the mice inoculated with 10^3 tachyzoites of the RH strain 5 or 6 days earlier. As shown in Fig. 1, no agglutination was observed in normal mouse sera, whereas clear

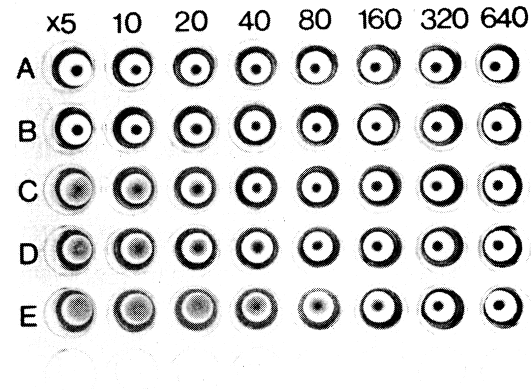

Fig. 1 Agglutination pattern of Ag-LA test. (A, B), normal mouse serum; (C, D, E), infected mouse serum. Ag-LA test titers are: (A, B) <1 : 5; (C) 1 : 20; (D) 1 : 40; (E) 1 : 160.

agglutination occurred in the infected mouse sera. The end points of the agglutination reactions were apparent, and the results of the reactions were reproducible.

Frequency of nonspecific reaction in normal mouse sera in Ag-LA test. Twenty-two normal ddY mice were bled, and the sera were subjected to Ag-LA test. No agglutination was observed in those sera except for one serum, in which a weak agglutination occurred only in the neat serum. These results indicate that there is little nonspecific reaction in Ag-LA test in mouse sera.

Change of Ag-LA and LA test titers during the course of primary Toxoplasma infection in mice. Mice were inoculated intraperitoneally with 10^6 tachyzoites of the RH strain, and were daily bled. The amounts of c-Ag and anti-*Toxoplasma* antibodies in sera were measured by Ag-LA and LA tests, repectively. As shown in Fig. 2, c-Ag became detectable from the 3rd day of infection. The Ag-LA titers increased rapidly by the 4th day of infection, and thereafter, gradually until death on day 6. Anti-*Toxoplasma* antibodies could be firstly observed on the 4th day of infection, and the antibody titers increased gradually. The antibodies were entirely sensitive to treatment with 0.1 M 2-ME.

When mice were inoculated intraperiton-

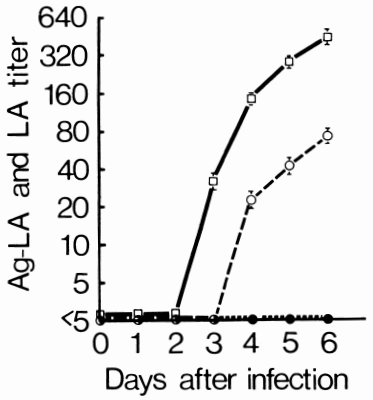

Fig. 2 Change of Ag-LA and LA test titers in mice during the course of infection with 10^6 tachyzoites of the RH strain of *T. gondii*. Each point represents the mean ± standard error of 8 mice. Symbols: □, Ag-LA test titer; ○, total LA test titer; ●, 2-ME-resistant LA test titer.

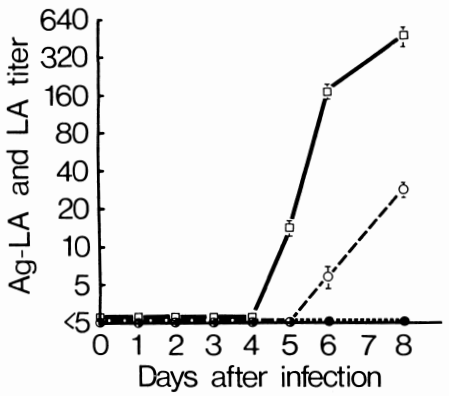

Fig. 3 Change of Ag-LA and LA test titers in mice during the course of infection with 10^3 tachyzoites of the RH strain of *T. gondii*. Each point represents the mean±standard error of 10 mice. Symbols: □, Ag-LA test titer; ○, total LA test titer; ●, 2-ME-resistant LA test titer.

eally with 10^3 tachyzoites, *Toxoplasma* antigens were detectable from day 5 of infection in the sera from the mice (Fig. 3). The Ag-LA test titer increased rapidly by day 6, and then still increased until the mice died. The antibodies to *Toxoplasma* organisms were observed from day 6 of infection, and the antibody titer increased linearly until day 8 on which the majority of mice died (Fig. 3). Also in this case, 2-ME resistant anti-*Toxoplasma* antibodies were not detected.

Change of Ag-LA and LA test titers in secondary Toxoplasma infection in mice. C57BL/6 mice were inoculated intraperitoneally with 5×10^3 bradyzoites of the avirulent Fukaya strain of *T. gondii*, and 3 months later, the mice were challenged with 10^5 tachyzoites of the RH strain intraperitoneally. As a control, normal C57BL/6 mice were inoculated with the 10^5 tachyzoites intraperitoneally.

In the primary infection, c-Ag was detected

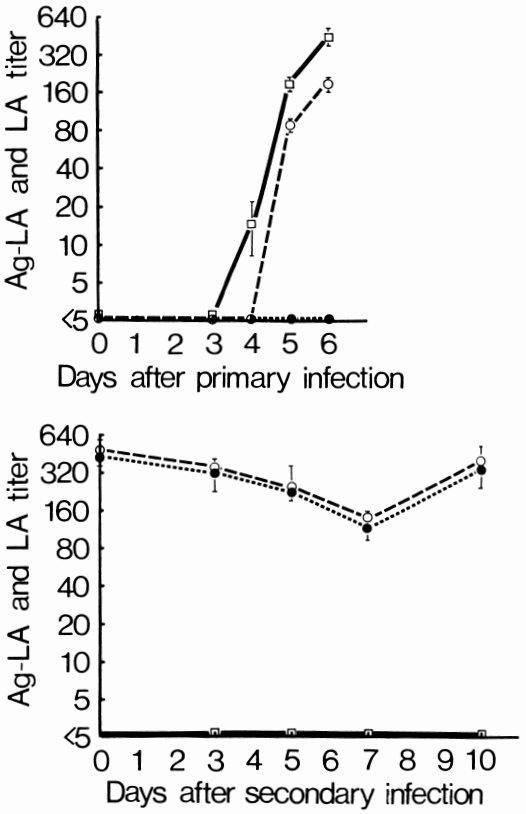

Fig. 4 Comparison of change of Ag-LA and LA test titers between primary and secondary *Toxoplasma* infections in mice. Normal and chronically *Toxoplasma*-infected mice were inoculated with 10^5 tachyzoites of the RH strain of *T. gondii*. Each point represents the mean± standard error of 5 or 6 mice. Symbols: □, Ag-LA test titer; ○, total LA test titer; ●, 2-ME-resistant LA test titer.

in sera from the infected mice on days 4, 5, and 6 of infection (Fig. 4). The Ag-LA test titer increased rapidly to 1 : 448 on day 6. All mice died on day 6 or 7. Anti-*Toxoplasma* antibodies were observed on the sera of days 5 and 6.

In the secondary infection, all mice died 12 or 13 days after the challenge infection with the virulent organisms. However, c-Ag was not detected throughout the observation period from day 3 to day 10 of challenge infection (Fig. 4). Anti-*Toxoplasma* antibody titer decreased gradually by day 7, and increased again on day 10 (Fig. 4). In the secondary infection, anti-*Toxoplasma* antibodies were resistant to 2-ME treatment.

Discussion

In the primary infection with the virulent RH strain of *T. gondii*, an appearance of c-Ag in the blood was earlier than that of anti-*Toxoplasma* antibodies during the course of infection. When mice were infected with 10^6 organisms, c-Ag were detectable on day 3 of infection, while anti-*Toxoplasma* antibodies were observed after the 4th day of infection. In the infection with 10^3 organisms, c-Ag appeared on the 5 th day of infection, and the antibodies could be detected after day 6. The detected antibodies were considered to be IgM, because they were sensitive to treatment with 0.1M 2-ME. These results indicate that c-Ag can be detected by Ag-LA test earlier than anti-*Toxoplasma* IgM antibodies became to be detectable during the course of infection. Raizman and Neva (1975) reported by the use of countercurrent electrophoresis and double-diffusion-in-agar that *Toxoplasma* antigens in sera are detected before the appearance of detectable antibody.

On the other hand, a large amount of c-Ag was detected by Ag-LA test even after anti-*Toxoplasma* antibody production started. On day 6 of infection with 10^6 organisms, anti-*Toxoplasma* LA test titer was 1 : 74, whereas Ag-LA test titer was 1 : 457. Similarly, on day 8 of 10^3 infection, LA and Ag-LA titers were 1 : 30 and 1 : 480, respectively. Therefore, it is conceivable that the detection of c-Ag by Ag-LA test would be highly useful for a rapid diagnosis in human cases of acute toxoplasmosis, since c-Ag became detectable earlier than anti-*Toxoplasma* IgM antibodies were observed, and after the start of antibody formation, a high concentration of c-Ag was detectable in mice during the course of *Toxoplasma* infection.

In the secondary *Toxoplasma* infection, c-Ag could not be detected although the mice died of acute toxoplasmosis. The reason for a failure of detection of c-Ag in the secondary infection is conceivable to be that anti-*Toxoplasma* IgG antibodies present in the blood bound to c-Ag to form immune complexes, thus, c-Ag could not be detected by Ag-LA test. The fact that anti-*Toxoplasma* IgG antibody titers decreased after the challenge infection will support this possibility. Siegel and Remington (1983) reported a presence of immune complexes in the sera of patients with toxoplasmosis.

A contrast in detection of c-Ag between the primary and secondary infections suggest a possibility that a quantitative assay of c-Ag by Ag-LA test can distinguish the primary from secondary *Toxoplasma* infection. In a clinical side, the distinction of both these infections is an important problem, because congenital *Toxoplasma* infection occurs only in the primary infection during the course of pregnancy (Remington and Desmonts, 1976).

The detection of c-Ag by Ag-LA test in human cases of acute toxoplasmosis is under study.

Summary

An appearance of *Toxoplasma* antigens in sera of the infected mice was measured by an agglutination test using anti-*Toxoplasma* antibody-coated latex particles. In the primary infections with the virulent RH strain of *T. gondii*, c-Ag could be detected earlier than an appearance of anti-*Toxoplasma* antibodies regardless of infectious doses of *T. gondii*. High concentrations of c-Ag were still detected by Ag-LA test after the start of a production of anti-*Toxoplasma* IgM antib-

odies. On the other hand, in the secondary infection with the virulent organisms, c-Ag was not observed although the mice died of acute toxoplasmosis. These results suggest that a detection of c-Ag by Ag-LA test would be available for a rapid etiological diagnosis in human cases of primary acute toxoplasmosis.

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抗トキソプラズマ抗体感作ラテックス粒子を用いた血中虫体抗原の検出について

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トキソプラズマ（Tp）感染にともない血中に出現する虫体抗原（c-Ag）を抗 Tp 抗体感作ラテックス粒子を用いた凝集反応（Ag-LA）により測定する方法について検討した．強毒株（RH 株）による一次感染マウスにおいては，感染量の多少（10^3，10^5，10^6 個）にかかわらず c-Ag は血中抗体の出現より早く検出され始めた．また，抗 Tp 抗体が検出されるようになった後も高濃度の c-Ag が Ag-LA 法により検出された．しかし，二次感染においては c-Ag は検出されなかった．以上の結果より，c-Ag の検出を目的とする Ag-LA 法は，初感染時の急性 Tp 症の診断に応用可能であると考えられた．