Presence of High Concentrations of Circulating *Toxoplasma* Antigens during Acute *Toxoplasma* Infection in Athymic Nude Mice

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In mice infected with an avirulent strain of *Toxoplasma gondii*, circulating *Toxoplasma* antigens were detectable in the sera during weeks 1 to 3 of infection by a simple agglutination test that uses latex particles coated with anti-*Toxoplasma* antibodies. An infection in athymic nude mice resulted in high agglutination titers in the anti-*Toxoplasma* antibody-coated latex particle test and the absence of anti-*Toxoplasma* antibodies in sera during the acute phase, suggesting that the detection of circulating *Toxoplasma* antigens is a good tool for the diagnosis of acute toxoplasmosis, especially in severely immunocompromised hosts.

In the serodiagnosis of acute toxoplasmosis, titration of anti-*Toxoplasma* immunoglobulin G antibodies by the Sabin-Feldman dye test and anti-*Toxoplasma* immunoglobulin M antibodies is important and informative (5, 16). However, these serological methods may be inconclusive or unreliable in patients with underlying diseases causing suppressed antibody responses or in patients receiving immunosuppressive therapy (6, 9, 17). In these cases, a direct detection of antigenic components of *Toxoplasma gondii* in sera or body fluids might be a valuable aid in the rapid and specific diagnosis of acute toxoplasmosis. Recently, it was reported that circulating *Toxoplasma* antigens (c-Ag) are detectable in sera from humans and animals with acute toxoplasmosis by countercurrent electrophoresis (8), enzyme-linked immunosorbent assay (1, 3, 14, 15), dot immunobinding (2), and radioimmunoassay (4). We have previously reported a simple agglutination test which uses latex particles coated with anti-*Toxoplasma* antibodies (Ag-LA) for the detection of c-Ag (10). However, there are no reports on the detection of c-Ag in immunosuppressed patients or animals. Therefore, in the present study, we examined the presence of c-Ag in congenitally immunodeficient nude mice infected with an avirulent strain of *T. gondii*.

Inbred female C57BL/6 mice were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Japan). Mice 6 to 10 weeks old were used in all experiments. BALB/c background nude (nu/nu) and heterozygote (nu/+ ) mice were purchased from the same breeder. For infection, bradyzoites of the avirulent Fukaya strain of *T. gondii* were obtained from the brains of chronically infected mice by treatment with 0.25% trypsin as described previously (12). Mice were inoculated with 1 × 10⁵ or 5 × 10⁵ organisms intraperitoneally. For the Ag-LA test for the detection of c-Ag, Immutex H1001 latex particles (Japan Synthetic Rubber Co. Ltd., Tokyo, Japan) were coated with rabbit anti-*Toxoplasma* immunoglobulin G antibodies by the method described by Tsutoba and Ozawa (13). A quantitative assay for c-Ag was performed by a microagglutination test with the Ag-LA. An equal volume (0.025 ml) of latex particle suspension at a concentration of 0.2% (wt/vol) was added to each of twofold dilutions of sera made in 0.2 M ammonium-buffered saline (pH 8.2) containing 0.1% bovine serum albumin in microtiter trays. The pattern of agglutination was read after the trays had stood at room temperature overnight. Anti-*Toxoplasma* antibodies were titrated by an indirect latex agglutination (ILA) test with a commercial kit product (Eiken Chemical Co. Ltd., Tokyo, Japan). In this test, titers of ≥1:16 are regarded as positive for anti-*Toxoplasma* antibodies in mice (11). For the preparation of *Toxoplasma* lysate antigens, the cell-free tachyzoites were lysed osmotically by suspension in distilled water, and a clear supernatant was obtained by high-speed centrifugation (10,000 × g for 30 min). The protein concentration of the lysate was measured by the method of Lowry et al. (7).

Prior to the examination of c-Ag in mice, we improved the sensitivity of the Ag-LA test over that in our previous study (10). The use of Immutex H1001 latex particles improved the sensitivity of the Ag-LA test 16-fold higher than that previously observed. The present Ag-LA test detected *Toxoplasma* lysate antigens in buffer at protein concentrations of ≥78 ng/ml. This sensitivity is nearly equal to that of the enzyme-linked immunosorbent assay (1). The frequency of nonspecific agglutination in normal mouse serum was very low. The Ag-LA titers of 37 normal C57BL/6 mice sera were 1:2 or less; therefore, we determined that sera with titers of ≥1:4 are positive for c-Ag in this test.

First, we examined whether c-Ag can be detected in mice infected with an avirulent strain of *T. gondii* by the Ag-LA test. C57BL/6 mice were infected with 5 × 10⁵ bradyzoites of *T. gondii* and bled at regular intervals. Figure 1 shows the changes in Ag-LA and ILA test titers during the course of infection. The Ag-LA test titers became positive on day 7 of infection, at which time anti-*Toxoplasma* antibodies could not be detected. The Ag-LA titers increased to 1:16 on day 10. After that time, the Ag-LA titers decreased gradually and became negative 28 days after infection. This demonstrates that c-Ag can be detected only in the acute phase of infection. On the other hand, anti-*Toxoplasma* antibodies became detectable on day 10 of infection, and high antibody titers were observed until day 28. These results indicate that the detection of c-Ag by the Ag-LA test is useful for the diagnosis of acute infection with *T. gondii*. In another experiment, c-Ag could be detected by the Ag-LA test in mice inoculated with only 2 × 10⁵ organisms (data not shown). Almost all of the studies on c-Ag in animal models

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which have been published have used the virulent RH strain of *T. gondii*; however, van Knapen et al. (15) recently reported that c-Ag were detected on day 14 of infection by an enzyme-linked immunosorbent assay in mice infected with the avirulent T626 strain of *T. gondii*. On the other hand, Brooks et al. (2) reported that c-Ag could not be detected in mice infected with the avirulent C56 strain of *T. gondii* by the dot-immunobinding technique. However, their observation period ended on day 5 of infection. It may have been possible to detect c-Ag in these mice after day 5 of infection.

As a model of acute toxoplasmosis in immunosuppressed patients, athymic nude mice were inoculated with 10⁵ bradyzoites of *T. gondii* and bled on day 14 of infection. As a control, heterozygote *nu*+/− mice were inoculated with *T. gondii* and bled in the same manner as the athymic *nu*/*nu* mice. The infected *nu*/*nu* mice showed more than fourfold-higher Ag-LA test titers than did the infected *nu*+/− mice (*P* < 0.001) (Table 1). In contrast, anti-Toxoplasma antibodies were detected in the infected *nu*+/− mice but not in the infected *nu*/*nu* mice. In uninfected control mice of both strains, Ag-LA test titers were very low. Since it has been reported that the diagnosis of acute toxoplasmosis by conventional serological methods which measure immunoglobulins G or M antibodies may be particularly difficult in severely immunocompromised patients with acquired immunodeficiency syndrome (17), these results suggest that the detection of c-Ag could be a good tool for the diagnosis of acute toxoplasmosis in severely immunosuppressed patients. The detection of c-Ag in human serum samples by the Ag-LA test is currently under study.

We thank K. Fuse, Eiken Chemical Co. Ltd., for his cooperation and C. M. Black and S. D. Sharma for kind advice in preparing this manuscript.

This work was supported by a grant-in-aid for scientific research (no. 59770293) from the Ministry of Education, Science and Culture, Japan.

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