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NOTES

High Correlation in Antibody Titers between the Sabin-Feldman Dye Test and an Enzyme-Linked Immunosorbent Assay Detecting Immunoglobulin G Antibodies to the Nucleoside Triphosphate Hydrolase of *Toxoplasma gondii*

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An enzyme-linked immunosorbent assay to detect immunoglobulin G antibodies against nucleoside triphosphate hydrolase, which is a specific and dominant antigen of *Toxoplasma gondii*, was developed, and the sensitivity and specificity of the test were compared with those of the Sabin-Feldman dye test. One hundred percent agreement was observed in comparative study between those tests on 37 positive and 50 negative human sera. Antibody titers in the enzyme-linked immunosorbent assay test, which were expressed as the reciprocal of the highest positive dilution of serum, were just 100 times those in the dye test on 81% (30 of 37) of the positive sera.

The most commonly recognized clinical manifestation of acute acquired infection with *Toxoplasma gondii* in immunocompetent hosts is lymphadenopathy (8). Although criteria for histopathologic diagnosis of toxoplasmic lymphadenopathy have been described (6), serologic diagnosis is preferable to having the patients undergo a biopsy (5). The Sabin-Feldman dye test (9) has been the standard of the serologic tests for diagnosis. However, the dye test is available in limited numbers of laboratories because of the requirement of live tachyzoites for the test. It would be helpful for diagnosis if we could develop a new serologic test which is equivalent to the dye test and which does not need live organisms.

We previously reported that nucleoside triphosphate hydrolase (NTPase) is one of the major antigens of tachyzoites of *T. gondii* (1, 3, 4) and is specific to the parasite (1, 3). We also reported that NTPase is detectable in sera of mice with acute infection (2). Therefore, NTPase appears to be one of the major antigens which stimulates antibody responses during the acute stage of infection. In the present study, we developed an enzyme-linked immunosorbent assay (ELISA) to detect anti-NTPase immunoglobulin G (IgG) antibodies (NTPase-ELISA) and found that antibody titers in NTPase-ELISA on patient sera were in high correlation with those in the dye test.

Sera were from 37 individuals with positive dye test titers ranging from 1:16 to 1:4,096, including four patients who had acute lymphadenopathic toxoplasmosis diagnosed by serology and histopathology (5, 7); five patients suspected of having it, although biopsy was not performed; and three patients who showed serologic test results suggesting acute acquired infection. Control sera were from 50 individuals whose sera were negative (<1:16) in the dye test.

The NTPase-ELISA test was performed as follows. A mouse monoclonal antibody to NTPase (2) was diluted with 0.1 M bicarbonate buffer (pH 9.6) to a concentration of 10 μg/ml. Wells of a polystyrene microtiter plate (Nunc, Roskilde, Denmark) were coated with 200 μl of the solution. After incubation at 37°C for 1 h, the wells were washed once with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS-Tween). The wells were postcoated with 250 μl of PBS containing 1% bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, Mo.) (PBS-BSA) at 37°C for 30 min. Thereafter, wells were again washed once in PBS-Tween. A crude NTPase preparation (30 mg of protein per ml) was diluted 1:1,000 with PBS-BSA, and the experimental wells were incubated at 37°C for 1 h with 200 μl of the antigen solution. Control wells were incubated with PBS-BSA instead of antigen solution. The wells were then washed four times at intervals of 10 min with PBS-Tween. Serial fourfold dilutions of serum sample (starting at 1:100) were prepared in PBS-BSA, and 200 μl of each dilution was transferred to both the control and the experimental wells. After incubation at 37°C for 1 h, the wells were washed four times with PBS-Tween. Subsequently, 200 μl of calf intestinal alkaline phosphatase-conjugated goat anti-human IgG (Cappel Inc., West Chester, Pa.) in PBS-BSA (final concentration, 1.1 U/ml) was added to each well, and the preparation was incubated at 37°C for 1 h. The wells were once again washed four times with PBS-Tween, and 200 μl of 1 M diethanolamine-HCl (pH 9.8) containing 0.2 mg *p*-nitrophe-

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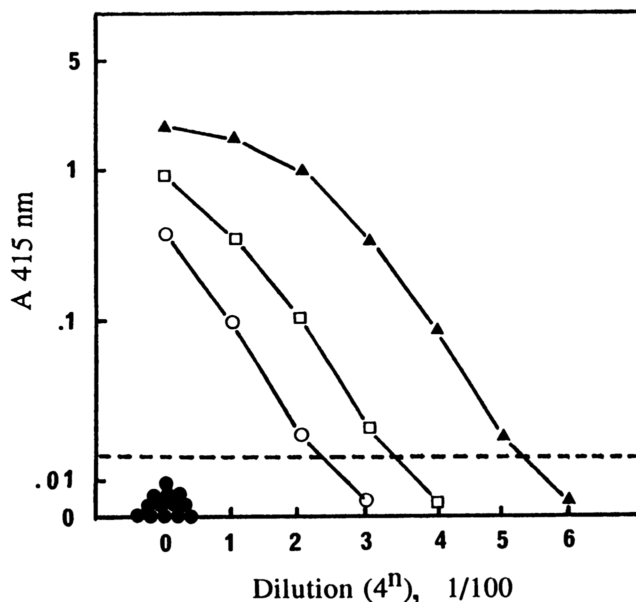

FIG. 1. Absorbances in NTPase-ELISA on dye test-negative and -positive sera. Symbols: ●, dye test-negative sera; ○, serum with a dye test titer of 1:16; □, serum with a dye test titer of 1:64; ▲, serum with a dye test titer of 1,024. The dotted line indicates the mean value + 3 SD of the absorbances in the negative group.

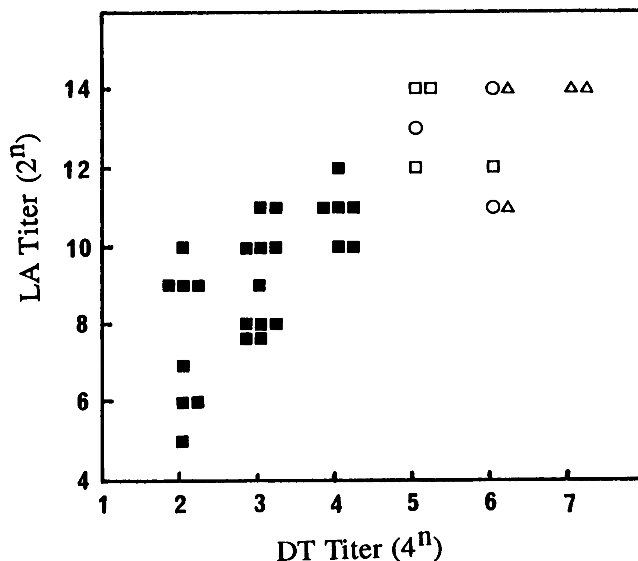

FIG. 3. Scatter plot for comparison of antibody titers measured in a latex agglutination test and the Sabin-Feldman dye test. Symbols: ■, patients with chronic infection of *Toxoplasma* sp.; △, patients with acute toxoplasmic lymphadenopathy; □, patients suspected of having acute toxoplasmic lymphadenopathy; ○, patients with serologic test results suggesting acute acquired infection.

nyl phosphate-2Na as substrate and 0.1 mg of $MgCl_2$ was added to each well. The reaction of the enzyme substrate was allowed to continue for 30 min, and optical density values at 415 nm were read with a microplate photometer (MTP-100; Corona Electric Co. Ltd., Ibaraki, Japan). The ELISA absorbance was expressed as the difference of optical density values between experimental and control wells on each serum. A latex agglutination test (Toxotest-MT; Eiken Chemical Co. Ltd., Tokyo, Japan) was performed as follows (directions are from a commercial insert). Briefly, 25 μl of the suspension containing latex particles coated with sonicated tachyzoite antigens was added to 25 μl of twofold dilutions of sera in microplates. Plates were read after overnight incubation at room temperature. Antibody titers in each serologic test were expressed as the reciprocal of the highest positive dilution of serum.

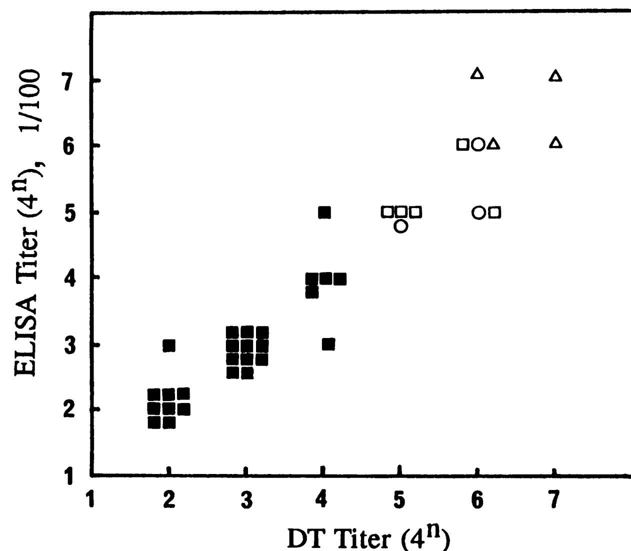

FIG. 2. Scatter plot for comparison of antibody titers measured in the NTPase-ELISA and the Sabin-Feldman dye test. Symbols: ■, patients with chronic infection of *Toxoplasma* sp.; △, patients with acute toxoplasmic lymphadenopathy; □, patients suspected of having acute toxoplasmic lymphadenopathy; ○, patients with serologic test results suggesting acute acquired infection.

As the first step of study, we applied 50 sera of the seronegative group to the NTPase-ELISA to determine whether those sera contain anti-NTPase IgG antibodies. The ELISA absorbances in the initial dilution (1:100) of those sera were very small and the mean value ± standard deviation of the values was 0.003 ± 0.004. On the basis of this result, we determined that the ELISA absorbances of greater than 0.015 (the mean value + 3 SD in the seronegative group) to be positive in dilutions of serum. All of the 50 sera from the seronegative group were negative in a 1:100 dilution (Fig. 1). It was determined that sera with the titers of ≥1:100 are positive in the NTPase-ELISA test.

In the next step, we applied 37 sera from the seropositive group to the NTPase-ELISA test. At a 1:100 dilution of serum for declaring serum positive, 33% (2 of 6) of sera having a dye test titer of 1:256 and all (12 of 12) of sera having titers of ≥1:1,024 showed absorbance values of >2, which is the upper limit of the absorbance range that the machine can measure. Changes in the ELISA absorbances in serial dilutions of sera from three representative patients with different dye test titers (1:16, 1:64, and 1:1,024) are shown in Fig. 1. The endpoint of positive reaction in serial dilution of each serum was quite clear. All of the 37 sera of the seropositive group showed positive results in the NTPase-ELISA test.

Correlation in antibody titers between NTPase-ELISA

and the dye tests is shown in Fig. 2. In 30 of 37 sera, antibody titers in the NTPase-ELISA were just 100 times those in the dye test. In the other seven sera, the ELISA test titers were in a range of one tube higher or lower in fourfold serial dilution than 100 times their dye test titers. The correlation coefficient between the titers in those two serologic tests was 0.921 in the linear regression analysis.

A latex agglutination test using latex particles coated with whole sonicated tachyzoite antigens was employed to compare with the dye test on 36 of the 37 sera of the seropositive group. Antibody titers in the latex agglutination tests did not correlate well with those in the dye test (Fig. 3). The correlation coefficient was 0.684. For example, the latex agglutination test titers on eight sera with the dye test titer of 1:16 were distributed in a wide range, from 1:32 to 1:1,024. These results, compared with those in NTPase-ELISA test, strongly suggest that selective use of NTPase from sonicated tachyzoite antigens is important to obtain a good correlation with results of the dye test.

As mentioned before, the dye test has been the standard of the serologic tests for diagnosis of toxoplasmosis. The titers of ≥1:1,024 in the dye test suggest acute infection in immunocompetent patients (5, 7). In the present study, all of four sera from the patients with acute toxoplasmic lymphadenopathy, five sera from the patients suspected of having the disease, and three sera of patients with serologic test results suggesting acute acquired infection showed antibody titers of ≥1:102,400 in NTPase-ELISA test. On the basis of this fact, with a high correlation in antibody titers between the dye test and the ELISA test, the NTPase-ELISA test appears valuable for serodiagnosis of acute toxoplasmosis as a simple method which does not require live organisms.

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