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Recognition of Tachyzoite and Bradyzoite Antigens of *Toxoplasma gondii* by Infected Hosts

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Western immunoblots of tachyzoite and bradyzoite antigens of *Toxoplasma gondii* were probed with antisera from rabbits and mice at intervals between 2 and 8 weeks after infection and with human antisera with various titers of antibody. With rabbit and mouse antisera, two groups of antigens with molecular masses of 54 to 63 kDa (designated 58.5-kDa antigens) and 26 to 29 kDa (designated 27.5-kDa antigens) were demonstrated commonly for both stages, while those antisera reacted strongly with tachyzoite (but not bradyzoite) antigens with molecular masses of 29 to 54 kDa. Tachyzoite antigens of 21.5, 26.5, 31, 38, 40, 49, and 58 kDa reacted with antisera 2 to 4 weeks after infection, while bradyzoite antigens of 27, 51, 220, and 290 kDa reacted with antisera obtained 4 or more weeks after infection. The 58.5-kDa antigens of both stages reacted primarily with human antisera that had low titers of anti-*T. gondii* antibodies. Human (as well as rabbit and mouse) sera with high antibody titers reacted with the 27.5-kDa antigens as well as the 58.5-kDa antigens, but the reactivity of the 27.5- to 58.5-kDa antigens of tachyzoites was greater than that of bradyzoites.

Extensive analyses of *Toxoplasma gondii* antigens recognized by infected hosts have been made to understand the host humoral immune response and also as a basis for serodiagnosis of toxoplasmosis. This has been done mainly with the RH strain of *T. gondii* as antigens for immunoblotting (9–12, 14). However, those analyses which used tachyzoites of the RH strain do not reflect natural infection, because tachyzoites are present early in infection and later there are primary encysted bradyzoites. Also, others have demonstrated the presence of stage-specific epitopes as well as shared *T. gondii* epitopes (2–5, 7, 8). It had been difficult to obtain large numbers of tachyzoites of avirulent strains of *T. gondii* to study both tachyzoite and bradyzoite antigens of the same strain of *T. gondii*. Suzuki and Remington (13) recently developed a method for obtaining large numbers of tachyzoites of avirulent strains of *T. gondii* by pretreatment of mice with monoclonal anti-gamma interferon antibody followed by infection with cysts of avirulent strains (ME49, C37, or DAG) of *T. gondii*. This produced large numbers of the tachyzoites in the peritoneal cavity. Among those strains, the ME49 strain is also suitable for obtaining bradyzoites because of production of large numbers of cysts in the brain of the infected mice (7).

These findings facilitated the present study, in which we probed Western immunoblots of tachyzoite and bradyzoite antigens of the ME49 strain of *T. gondii* with rabbit, mouse, and human antisera elicited by *T. gondii* infection and compared which epitopes were recognized.

MATERIALS AND METHODS

Animals and parasites. Female outbred ddY and BALB/c *nu/nu* mice (6 to 8 weeks old) were used for the preparation of *T. gondii* antigens and of monoclonal antibody, respectively. Female C57BL/6 mice (6 to 8 weeks old) and rabbits (adult) were used for infection. All of the animals were obtained from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan. Two avirulent strains of *T. gondii*, ME49 and Fukaya, were used for infection. The ME49 strain was kindly provided by J. S. Remington, Palo Alto Medical Foundation, Palo Alto, Calif., and the Fukaya strain has been maintained in our laboratory.

Preparation of tachyzoite and bradyzoite antigens. Tachyzoites of the ME49 strain of *T. gondii* were prepared as described previously (13). Ascitic fluid containing monoclonal anti-gamma interferon antibody was obtained from mice injected with hybridomas (kindly provided by S. Waki, Gunma University, Maebashi, Japan) secreting the antibody, and the antibody was purified by ammonium sulfate precipitation. ddY mice were injected intraperitoneally (i.p.) with 100 μg of the antibody and 1 day later were inoculated i.p. with 10 cysts of the ME49 strain of *T. gondii*. Seven days later, mice were sacrificed and their peritoneal fluids containing tachyzoites were harvested and passed through a 27-gauge needle and a 3-μm-pore-size polycarbonate membrane filter (Nuclepore Corp., Pleasanton, Calif.) to remove host cells. Cysts of the ME49 strain of *T. gondii* were separated from the brains of chronically infected mice as described previously (1). Briefly, infected brains were homogenized in phosphate-buffered saline (PBS) and the brain suspension was mixed with a 45% Percoll suspension in PBS and centrifuged at 27,000 × *g* for 20 min. Cysts were recovered in the top half of the gradient, resuspended in PBS, and centrifuged at 150 × *g* for 10 min. Bradyzoites were obtained by digestion of about 1,500 cysts for 1 min in 1 ml of pepsin (0.26%)–HCl and then centrifuged in PBS at 2,000 × *g* for 10 min.

Electrophoresis and electrophoretic transfer. Purified tachyzoites and bradyzoites were solubilized in sample buffer and subjected to electrophoresis; 10^5 organisms (each type) per lane were used. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with a Tris-glycine buffer, pH 8.3, containing 0.1% SDS, as described by Laemmli (6), on 12.5% polyacrylamide slab gels. The proteins separated by SDS-PAGE were then transferred to

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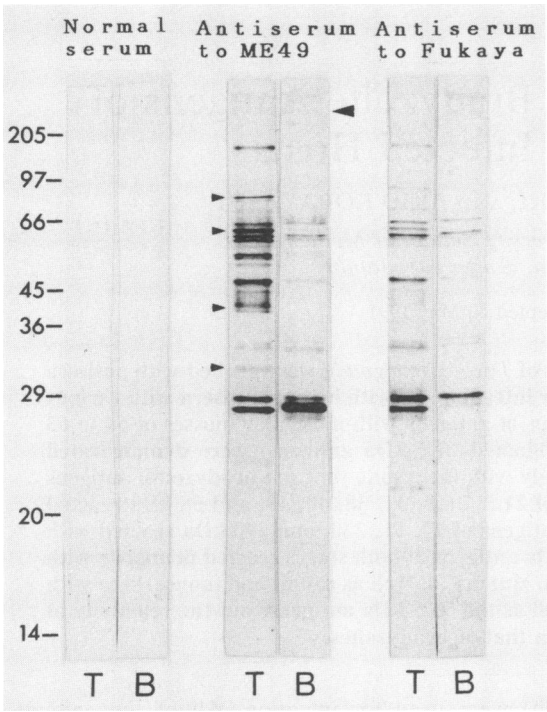

FIG. 1. Immunoblot analysis of tachyzoite and bradyzoite antigens of *T. gondii* reacted with normal rabbit serum or antiserum from two rabbits infected with the ME49 or Fukaya strain. The figure shows representative results from two analyses. Equal numbers (10^5 per lane) of purified tachyzoites (T) and bradyzoites (B) were subjected to SDS–12.5% PAGE, transferred to nitrocellulose paper, and assayed for immunoglobulin G binding. Arrowheads on the left and right of the center lanes indicate tachyzoite-specific and bradyzoite-specific antigens, respectively. Numbers on the left indicate molecular masses in kilodaltons.

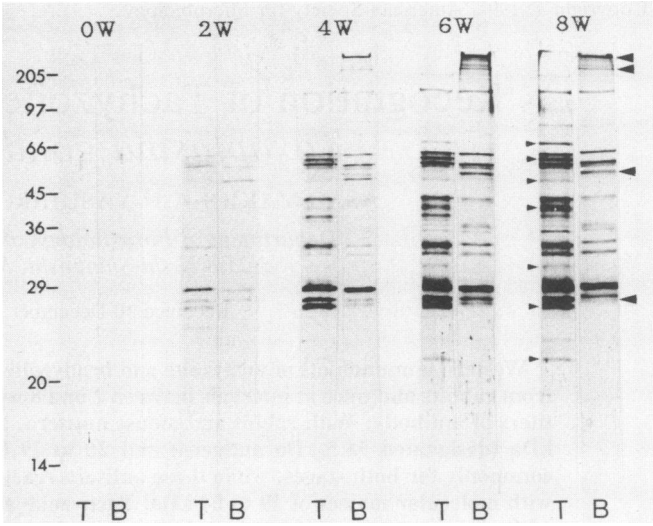

FIG. 2. Immunoblot analysis of tachyzoite (T) and bradyzoite (B) antigens reacted with antisera from a rabbit infected with the ME49 strain during the course of infection. The rabbit used differs from ones used for Fig. 1, and the results are representative from two analyses. Conditions for SDS-PAGE and transfer were the same as for Fig. 1. Arrowheads (small and large) indicate tachyzoite-specific and bradyzoite-specific antigens, respectively. W, weeks. Numbers on the left indicate molecular masses in kilodaltons.

nitrocellulose paper with 25 mM Tris-HCl buffer, pH 8.3, containing 192 mM glycine and 20% (vol/vol) methanol, with a commercial blotting apparatus (ATTO Co., Tokyo, Japan).

Immunoblotting. For immunoblotting, nitrocellulose strips were soaked for 30 min in PBS containing 10% (vol/vol) fetal bovine serum and 0.2% (vol/vol) Triton X-100 (PBS/FBS/TrX), washed, and then exposed to 1:100-diluted rabbit, mouse, and human sera in PBS/FBS/TrX for 30 min. After washing, the nitrocellulose strips were incubated for 30 min with peroxidase-conjugated goat anti-rabbit, -mouse, or -human immunoglobulin (Cappel Laboratory) diluted 1:100 in the same buffer as above. After washing, the strips were developed with 3,3-diaminobenzidinetetrahydrochloride and H_2O_2 (9). For experimental infection of rabbits and mice with *T. gondii*, brains from mice chronically infected with the ME49 or Fukaya strain were removed and homogenized in PBS. The number of cysts was counted, and 10 cysts of each strain were injected i.p. into rabbits and mice. Those mice and rabbits were bled sequentially. The human antisera used were obtained from patients who were investigated at Jikei University Hospital, Tokyo, Japan. Anti-*T. gondii* antibody was measured by an indirect latex agglutination test (LA) using a commercial kit (Toxo-MT; Eiken Chemical Co., Tokyo, Japan). Human sera with LA titers of 1:4,096 and 1:32,000 were obtained from the same person, and other sera with LA titers of <1:2 (negative), 1:64, and 1:1,024 were from different persons.

RESULTS

To compare the tachyzoite and bradyzoite epitopes recognized by sera from infected rabbits, immunoblotting was performed using sera from uninfected rabbits and from a rabbit infected with the ME49 strain 2 months earlier. The results are shown in Fig. 1. Groups of antigens of 54 to 63 kDa (designated 58.5-kDa antigens) and 26 to 29 kDa (designated 27.5-kDa antigens) for both stages reacted commonly with the antiserum to the ME49 strain. Bradyzoite antigens of 29 to 54 kDa reacted less than did those of tachyzoites. Also, tachyzoite-specific antigens were more often observed than bradyzoite-specific ones. To evaluate the antigen recognition of both stages by rabbit antiserum to different strains of *T. gondii*, antiserum from a rabbit infected 2 months before with the Fukaya strain was used for immunoblotting. These results are also shown in Fig. 1. In this case, reactive antigens of both stages were present in both the ME49 and Fukaya strains. The 58.5- and 27.5-kDa antigens of both stages reacted mainly with the antiserum to Fukaya strain, while bradyzoite antigens of 27.5 to 58.5 kDa reacted poorly compared with those of tachyzoites. This immunoblot pattern was similar to that of the ME49 strain.

We next examined the immunoblot patterns of both stages during the course of infection. For this, a rabbit was infected i.p. with the ME49 strain and antisera were obtained by bleeding just before infection and at 2, 4, 6, and 8 weeks postinfection. The results are shown in Fig. 2. A sharp increase in the reactivities of common antigens of 27.5, 28.5, 33, 56, 63, and 130 kDa to both stages and tachyzoite-specific antigens was observed at from 2 to 4 weeks postinfection, while bradyzoite-specific antigens of 27, 51, 220, and 290 kDa reacted with the antisera of 4 weeks and after.

Recognition of antigens to both stages by a mouse infected with *T. gondii* was compared with that by a rabbit. A C57BL/6 mouse was infected i.p. with the Fukaya strain, and sera were obtained by bleeding just before infection and

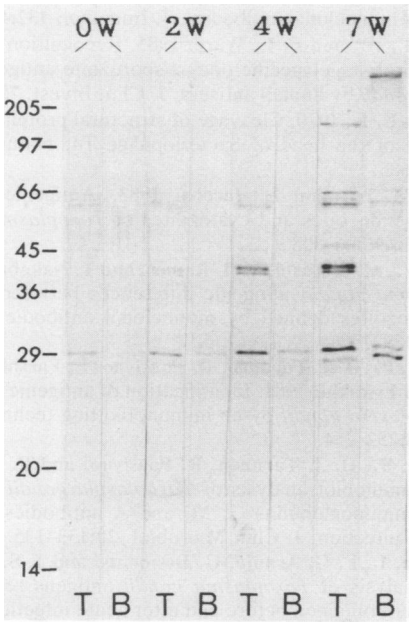

FIG. 3. Immunoblot analysis of tachyzoite (T) and bradyzoite (B) antigens reacted with antisera from a mouse infected with the Fukaya strain during the course of infection. The figure shows representative results from two analyses. Conditions for SDS-PAGE and transfer were the same as for Fig. 1. W, weeks. Numbers on the left indicate molecular masses in kilodaltons.

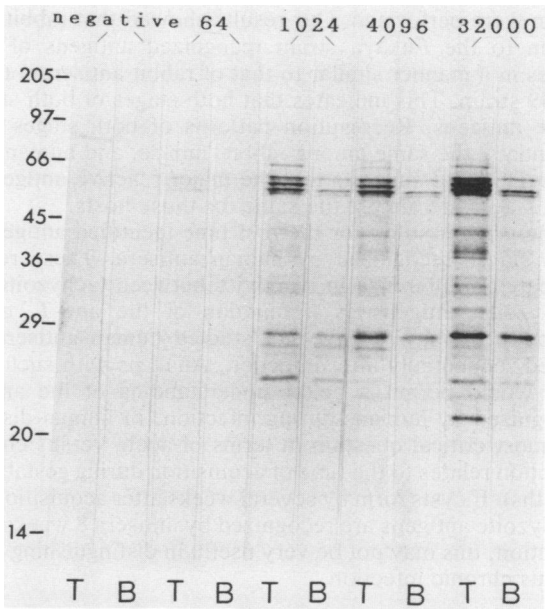

FIG. 4. Immunoblot analysis of tachyzoite (T) and bradyzoite (B) antigens reacted with human antisera. The figure shows representative results from two analyses. Conditions for SDS-PAGE and transfer were the same as for Fig. 1. Human antisera (1:100 diluted) having the LA titers of <1:2 (negative), 1:64, 1:1,024, 1:4,096, and 1:32,000 were used. Numbers on the left indicate molecular masses in kilodaltons.

at 2, 4, and 7 weeks after infection. As shown in Fig. 3, 58.5- and 27.5-kDa antigens to both stages were the main reactive antigens. Reactivity of bradyzoite antigens of 27.5 to 58.5 kDa was weaker than that of tachyzoite antigens. This observation was almost the same as for the rabbit infected with the Fukaya strain, although the reactivity of the mouse antiserum was weaker than that of the rabbit antiserum. Also, the increase in reactivity of the 58.5-, 27.5-, and 27.5- to 58.5-kDa antigens of tachyzoites was observed at 2 to 4 weeks, while that of the 58.5- and 27.5-kDa antigens of bradyzoites was observed at 4 to 7 weeks.

To elucidate the recognition pattern of antigens of both stages by human serum, immunoblot analysis was made using human antisera with LA titers of <1:2 (negative), 1:64, 1:1,024, 1:4,096, and 1:32,000. The results are shown in Fig. 4. The 58.5-kDa antigens of both stages mainly reacted with the antiserum with a low titer (LA; 1:64) of anti-*T. gondii* antibody. With an increase in the antibody titer, the 27.5-kDa antigens as well as the 58.5-kDa ones of both stages became reactive. The reactivity of those bradyzoite antigens of 27.5 to 58.5 kDa was weak compared with that of tachyzoites. This immunoblot pattern was essentially similar to those of rabbit and mouse. However, the human antisera did not appear to detect the higher-molecular-weight bands shown in the bradyzoite antigens which were detected when rabbit antisera probed blots.

DISCUSSION

In *Toxoplasma* infection in humans and other animals, tachyzoites are present in the acute phase and encysted bradyzoites are present in the chronic phase. In addition, the presence of stage-specific antigens as well as common ones among stages of *T. gondii* has been reported (2–5, 7, 8). These considerations and also the development of a new method for obtaining a large number of tachyzoites from avirulent strains of *T. gondii* led us to compare the immunoblot patterns of tachyzoite and bradyzoite antigens by using antisera from infected hosts.

More than 10^6 RH strain tachyzoites per lane on SDS-PAGE for immunoblotting have usually been used, but in the present study 10^5 tachyzoites or bradyzoites per lane were used because of limited recovery of parasites of both stages.

Our first immunoblot analysis was to examine the recognition pattern of both stages by rabbit antiserum to the ME49 strain. The results indicated that the antiserum reacted with two groups of antigens of 58.5 and 27.5 kDa to both stages, while bradyzoite antigens of between 58.5 and 27.5 kDa reacted poorly compared with those of tachyzoites. In this regard, it has to be considered that unlike tachyzoites, bradyzoites exist within cysts and so only antigens on the cyst wall or passed through it may be recognized by the host. Thus, it is likely that lower exposure of bradyzoite antigens to the host results in reduced antigen recognition and antibody production. Kasper (3) reported the presence of stage-specific tachyzoite and bradyzoite antigens as well as common antigens by using antisera to sonicated and freeze-thawed parasites of each stage. Also, Darcy et al. (2) have identified and characterized commonly shared antigens of both stages. In the present study we have also confirmed the presence of stage-specific antigens, but our results are not directly comparable with those of previous studies because of our use of antisera produced by infection rather than by immunization.

In addition to the presence of stage-specific antigens of *T. gondii*, there exists antigenic variation among strains of *T. gondii* (15). In the natural infection, it is difficult to determine the infecting isolate of *T. gondii*. Therefore, immunoblot analyses using antisera to strains different from the ME49

strain were performed. Our results showed that rabbit antiserum to the Fukaya strain recognized antigens of both stages in a manner similar to that of rabbit antiserum to the ME49 strain. This indicates that both stages of both strains share antigens. Recognition patterns of both stages were essentially the same among rabbit, mouse, and human antisera. This also suggests that the major reactive antigens of both stages are almost the same for those hosts.

The present study for the first time identified antigens of both stages recognized by human antisera. These results indicated a difference in reactivity between tachyzoite and bradyzoite antigens as a function of the anti-*T. gondii* antibody titers. We have not studied human antisera obtained sequentially after infection. Analysis with such antisera would permit a better understanding of the antigen recognized by humans during infection. In human disease, the most critical question in terms of acute versus chronic infection relates to the time of acquisition during gestation (9 months). If cysts form by several weeks after acquisition and bradyzoite antigens are recognized by antisera 8 weeks after infection, this may not be very useful in distinguishing acute versus chronic infection.

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