Evaluation of antigenic variations between two virulent toxoplasma strains

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Toxoplasma gondii infection in humans is routinely assessed by serological means. Here, the authors attempted to compare the response of different *Toxoplasma* strains to serological tests and to evaluate the antigenic profiles of the RH and RH Ankara (TRH) strains with Western blotting. Anti-*Toxoplasma* IgG antibodies of 72 patients were examined with the indirect immunofluorescence antibody (IFA) test, ELISA and Western blotting (WB) by using antigen from both strains. Antigenic variations between strains did not affect IFA and ELISA test results, but qualitative and quantitative differences between the WB patterns were observed. A number of bands with molecular masses varying between 17 and 105 kDa were detected in WB. Fourteen different bands were obtained with the assay performed with RH strain antigen. An additional four bands were observed with TRH strain antigen. Also, an 80 kDa band was observed to stain darker in the blot with TRH strain antigen, whereas with RH strain antigen 30 and 38 kDa bands were darker. The results showed that strain-specific polymorphism in tachyzoite antigens of different *Toxoplasma* strains is important in the evaluation of WB but not in conventional serological analyses such as ELISA and IFA.

Received 8 May 2006

Accepted 14 June 2006

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite capable of infecting all species of mammal. It is found worldwide with a large range of clinical manifestations and high prevalence, which indicates that it is one of the most successful parasites of man. Serious disease due to various parasite strains occurs in congenitally infected children and in immunocompromised patients; in particular, in the case of HIV infection (Hill *et al.*, 2005; Montoya & Liesenfeld, 2004). Not only the severity of toxoplasmosis, but also the immune response against it, is partly determined by parasite strain (Boothroyd & Grigg, 2002; Rodgers *et al.*, 2005; Saeij *et al.*, 2005).

The presence of different strains of *T. gondii* with different antigenic characteristics has been demonstrated by detailed studies with mAb techniques, Western blotting, isoenzyme analyses, RFLP, and random amplified polymorphic DNA PCR (RAPD-PCR) (Ware & Kasper, 1987; Kong *et al.*, 2003; Darde *et al.*, 1992; Darde, 2004; Ajzenberg *et al.*, 2005).

In this study, we aimed to evaluate possible strain-dependent variations of two different *Toxoplasma* strains, RH and RH Ankara, in the detection of anti-*Toxoplasma*

IgG antibodies by ELISA, and indirect immunofluorescence antibody (IFA) and Western blotting (WB) techniques.

METHODS

Subjects. The study group for investigation of anti-*Toxoplasma* IgG antibodies consisted of 72 patients with suspicion of toxoplasmosis who presented to the Adnan Menderes University Medical Faculty Parasitology Department in 2001–2002. Ten healthy subjects negative for toxoplasma infection were used as negative controls in serological tests. Approximately 6 ml venous blood was taken from each patient and centrifuged for separation of serum. Sera were kept at –20 °C until the serological tests were performed. Using antigens from each strain, in-house ELISA, IFA and WB tests were performed for each serum sample separately.

Antigens. *T. gondii* antigens were prepared from tachyzoites of the RH and RH Ankara (TRH) strains. The TRH strain, which was isolated from mice infected with intestinal extracts of stray cats in Ankara city, is considered to be the main strain of toxoplasmosis in Turkey. This strain belongs to the type 1 group, since tachyzoites of the strain kill intraperitoneally infected mice in 4–6 days (Degerli *et al.*, 2003; Doskaya *et al.*, 2006).

For antigen preparation, tachyzoites of the RH and TRH strains were harvested from the peritoneal cavities of previously infected BALB/c mice. After washing three times in PBS, the tachyzoites were used as antigen in an IFA test (Ertug *et al.*, 2000a). After dilution at a concentration of 10^5 ml⁻¹ in PBS, tachyzoites were fragmented with 1 % SDS and centrifuged at 10 000 r.p.m. and 4 °C for 30 min. The supernatants were used as antigen in ELISA and WB tests.

Abbreviations: IFA, indirect immunofluorescence antibody; WB, Western blotting.

ELISA. In-house ELISA was performed as described previously (Ertug *et al.*, 2000a, b). The optimal concentration of the antigens for ELISA was determined using the checkerboard method. Each serum was examined twice. Results were determined for each serum by calculating the mean A_{405} value for duplicate wells. A positive result was estimated as any value higher than three standard deviations above the mean A_{405} obtained from the 10 sera of the negative control serum group.

IFA test. The in-house IFA test was performed as described previously (Ertug *et al.*, 2000a). Briefly, serial serum dilutions starting from 1:16 were prepared in PBS and dropped on wells of microscope slides covered with *T. gondii* tachyzoites. Fluorescein-labelled anti-human IgG (Sigma) was used as conjugate. Results were evaluated under the fluorescence microscope.

Immunoblotting. Soluble antigen of each strain was fractionated by SDS-PAGE and the in-house WB test was performed as described previously (Ertug *et al.*, 2000b). Briefly, following separation (10% separation gel, 5% stacking gel), *T. gondii* antigens (RH or TRH) were transferred electrophoretically onto 0.45 µm pore-size nitrocellulose membranes, blocked with 3% casein and cut into strips. Strips were incubated for 1 h with sera and diluted 1/100 in Tris-buffered saline, and protein recognition by the sera was revealed by incubation with anti-human IgG-alkaline phosphatase conjugate (Sigma) and then with the chromogenic substrate nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BICP). The molecular masses of the bands were evaluated by comparison with a molecular mass marker (Bio-Rad) that was processed simultaneously.

RESULTS AND DISCUSSION

Of the 72 patients, 36 had anti-*T. gondii* IgG antibodies, detected by ELISA and IFA tests prepared with TRH strain antigen, and in addition these 36 patients had similar results with the tests performed with RH strain antigen. Twenty-five patients had low titres of antibody (IFA IgG ≤ 1/128; ELISA IgG ≤ 1/1024) and 11 had high titres of antibody (IFA IgG > 1/128; ELISA IgG > 1/1024). No antibody responses were detected with TRH or RH strain antigen in the remaining 36 patient sera. All seropositive patients detected with ELISA and IFA tests also had IgG responses with WB. Similarly, seronegative patients did not show an antigenic response with WB.

The WB patterns obtained with TRH and RH strain antigens differed substantially. Fourteen different bands were obtained by using the RH strain antigen, ranging from 17 to 105 kDa (17, 22, 25, 30, 32, 34, 35, 38, 43, 48, 60, 70, 80 and 105 kDa). Each serum from seropositive patients recognized 8–14 of these proteins. When TRH strain antigen was used in WB, four additional bands (41, 52, 54 and 58 kDa) were observed. While 30 and 38 kDa molecular mass bands were darker in the blotting with the RH strain, an 80 kDa molecular mass band was darker with the TRH strain. Representative bands of WB with RH and TRH antigens are shown in Fig. 1.

In biological and epidemiological studies, three main genotypes are generally recognized in the *T. gondii* population, types 1, 2 and 3 (Saeij *et al.*, 2005; Darde *et al.*, 1992; Howe & Sibley, 1995). From a phylogenetic point of view, types 2 and

Fig. 1. Representative IgG bands of sera from four patients (P-1 to P-4) in WB with RH and TRH antigens. *Additional bands seen with TRH antigen.

3 belong to the same clonal group. Findings in a study of 106 isolates have suggested that type 1 isolates are significantly more often associated with human congenital toxoplasmosis than with animal infection or reactivation of chronic infections in patients with AIDS (Howe & Sibley, 1995). When the virulent *Toxoplasma* strains are investigated according to phenotypic properties, significant differences are found between strains, which suggests that genetic variations can be found within this group (Appleford & Smith, 1997). All the *Toxoplasma* strains are morphologically similar, and when the immune responses are examined serologically, all of them give rise to the same pattern of response (Howe & Sibley, 1995; Dubey, 1998). In this study, we observed that the use of antigens of two different strains did not affect the results of ELISA and IFA tests. Strain-specific antigenic variability among various strains of *T. gondii* has been investigated by WB (Ware & Kasper, 1987; Weiss *et al.*, 1988; Appleford & Smith, 2000). Eight antigenic bands ranging between 26 and 105 kDa have been reported as important in differentiation of *Toxoplasma* strains. Bands of molecular masses 55, 71 and 130 kDa have been observed only with the RH strain, whereas bands of 24 kDa with the C₅₆ strain, and 21 and 91 kDa with the RH and T₁₀₀ strains, have been observed. Also, the detection of different antigenic bands in two different toxoplasmosis outbreaks has shown that different strains of *T. gondii* caused the outbreaks (Weiss *et al.*, 1988). In this study, 14 different bands of molecular masses ranging from 17 to 105 kDa were

obtained by using RH strain antigen. When TRH strain antigen was used in WB, three additional bands with molecular masses ranging between 48 and 60 kDa were observed. Findings in an earlier study that included three different strains revealed that 30–33, 21 and 25 kDa molecular mass bands were common to all the strains, but qualitative and quantitative differences were detected (Appleford & Smith, 2000). In our study, the 30 kDa molecular mass band was darker in the blotting with the RH strain antigen, similar to the results of Appleford & Smith (2000). However, with the TRH strain antigen, an 80 kDa molecular mass band was observed to be darker than the other bands.

It has been reported that as the antibody titres increase, the darkness of the bands seen in WB also increases (Ware & Kasper, 1987; Cazabonne *et al.*, 1994). We also observed that, as antibody response increased in conventional serologic tests, the number and darkness of the bands seen in WB increased. A further study with serological follow up of seropositive subjects could more clearly illustrate a direct correlation between antibody response and qualitative and/or quantitative changes in WB.

In conclusion, the employment of antigens from the two different strains did not affect the results of ELISA or IFA tests, because of the common antigenic epitopes. On the other hand, the demonstrated differences in antigenic profiles suggest that WB is a valuable test for differentiation of *Toxoplasma* strains. Further analysis of the antigenic determinants recognized by humoral and cellular immune responses may have important implications for diagnosis, therapy and vaccination strategies.

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