Serological reactivity against cyst and tachyzoite antigens of *Toxoplasma gondii* determined by FAST-ELISA

Y W Zhang, A Fraser, A H Balfour, T G Wreghitt, J J Gray, J E Smith

Abstract

Aims—To obtain quantitative data on the human serological response to *Toxoplasma gondii* tachyzoite and bradyzoite antigens.

Methods—Serum samples from 30 patients who had positive antibody titres against *T. gondii* and from 14 who were seronegative, together with sequential serum samples from four infected individuals, were screened by FAST-ELISA.

Results—Serum samples from the 30 seropositive patients showed high IgG and IgM titres against the *T. gondii* tachyzoite antigen but very low responses to cyst antigen. This result was borne out in sequential serum samples from patients with toxoplasmosis.

Conclusion—Antibody recognition of the cystic stage of *T. gondii* is low, implying that either this stage is poorly immunogenic or that the antigen load is low.

Keywords: FAST-ELISA, *Toxoplasma gondii*, tachyzoite antigen, cyst antigen.

The intracellular protozoan parasite *Toxoplasma gondii* is an important mammalian pathogen. It is geographically widespread, and is thought to infect approximately one third of the world’s human population, although the prevalence of the parasite varies from country to country. The majority of human infections are acquired postnatally by ingestion of tissue cysts in meat or of resistant oocysts from contaminated cat faeces. Infection of the host by *T. gondii* leads to an acute systemic phase, characterised by the presence of motile proliferative tachyzoites. The acute phase commonly lasts two to three months in human patients and is followed by a chronic phase, in which “dormant” bradyzoites within cysts are found in the skeletal muscles and central nervous tissue.

In immunocompetent hosts parasite proliferation during the acute phase of the infection is effectively controlled by a number of host immune response effector systems. The major mediators of resistance to *T. gondii* are parasite specific, cytotoxic T lymphocytes, which are involved in the destruction of tachyzoites, and interferon γ (IFN-γ), which has a number of immunostimulatory and parasitostatic effects. In addition, *T. gondii* specific antibodies have also been implicated in control of the acute phase of the infection. IFN-γ is thought to inhibit the spread of the parasite in the brain.

Immune surveillance of the cystic stage is important for parasite control and prevention of recrudescence. Although serological responses to tachyzoite antigens have been previously studied in patients during infection, the humoral immune response to the cystic stage is poorly documented. A number of studies have revealed that the tachyzoite and bradyzoite stage are antigenically distinct, and stage specific antigens exist. Furthermore, western blotting studies with human serum have shown that relatively few cyst antigens are recognised. However, quantitative estimates of cyst antibodies have not been made in humans. We have previously compared the reactivity of murine serum samples to tachyzoite and cyst antigens by the Falcon assay screening test (FAST/enzyme linked immunosorbent assay (ELISA)), and have found that the antibody response is predominantly directed against the tachyzoite. In the present study we have used the same technique to compare human serological responses to tachyzoite and cyst antigen.

Methods

*T. gondii* antigen used throughout the study was prepared from RH strain tachyzoites and RRA (Beverley) tissue cysts. Both strains were routinely maintained by serial passage in adult female outbred mice (Tucks No. 1). Tachyzoites were produced by inoculation of mice with 5 x 10⁶ tachyzoites intraperitoneally and collection of parasites three to five days later by peritoneal lavage in phosphate buffered saline (PBS). Tissue cysts were harvested from infected mouse brain approximately eight to 12 weeks after infection with 30 cysts per os and purified through 45% Percoll gradients according to the method of Cornelissen *et al.*

Soluble antigen extracts were prepared from tachyzoites and cysts for use in the ELISA. Parasite suspensions were freeze-thawed three times, sonicated on ice five times for 10 seconds at five minute intervals and then left at 4°C overnight. The supernatant fluids were collected following centrifugation at 11 000 x g for 60 minutes at 4°C and the protein concentration of the antigen preparation determined using the Bradford protein assay.

A panel of human serum samples was supplied by the Toxoplasma Reference Laboratory, Leeds Public Health Laboratory, from patients referred for routine screening. All serum samples had previously been tested using the
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A panel of 30 serum samples with positive dye test titres (16–64 000) and 14 serum samples with negative dye test titres (<8) were selected for use. Four sets of sequential serum samples were also analysed. Two were obtained from immunosuppressed heart transplant recipients with reactivation of a previous *T. gondii* infection. These two patients did not have severe symptoms and did not receive specific anti-toxoplasma treatment. A further two sets of sequential serum samples were taken from laboratory workers who developed asymptomatic toxoplasmosis following accidental infection with the RH strain.

The FAST-ELISA system (Becton Dickinson, Oxnard, California, USA) was used to detect specific antibodies to *T. gondii*. This system consisted of a lid holding 96 beads in a 12×8 configuration and ELISA plates for serum incubation and colour development. Tachyzoite or cyst antigen was diluted to 4 g/ml with antigen sensitisation buffer (0-05 M Tris/HCl, 0-3 M KCl, 2 mM ethylenediaminetetraacetic acid, pH 8-0) and coated onto the beaded lids overnight at 4°C. The lids were then washed with PBS containing 0-1% Tween 20 (PBS-TW), rinsed with PBS and air dried at room temperature for a minimum of six hours. The lids were sealed in plastic bags and stored at 4°C until use.

Antigen coated beads were incubated with the human serum samples, diluted 1 in 160 with PBS-TW plus 1% bovine serum albumin (BSA) for one hour at room temperature, and washed three times with PBS-TW. Antibody binding was detected by incubation for one hour at room temperature with either rabbit anti-human IgG peroxidase conjugate (Dako, High Wycombe, UK) diluted 1 in 3000 with PBS-TW, or goat anti-human IgM peroxidase conjugate diluted 1 in 1000 (Sigma, Poole, Dorset, UK). The lids were then washed as before and bound antibody was visualised using TMB (tetramethylbenzidine) peroxidase substrate (Kirkegaard & Perry Laboratories Inc., Gaithersburg, Maryland, USA). Absorbance was measured at 630 nm using a Titertek Multiskan ELISA plate reader. Serum samples with an optical density (OD) greater than twice the OD of the pooled negative control were considered positive.

**Results**

We initially compared the IgG response to tachyzoite and bradyzoite antigen in a panel of 30 dye test positive and 14 dye test negative human serum samples using FAST-ELISA (fig 1). We noted that the binding response of serum samples from infected patients to tachyzoite antigen was much higher (1.242 ± 0.087) than to cyst antigen (0.325 ± 0.021). All negative serum samples exhibited low recognition of both tachyzoite (mean OD 0.040 ± 0.004) and cyst antigen (mean OD 0.085 ± 0.015).

The results obtained with sequential serum samples taken from heart transplant recipients reinforce the pattern seen with the panel of *T. gondii* seropositive patients in that cyst re-
Discussion

The present study confirms previous qualitative immunoblotting data in demonstrating that patient responses to cyst antigens during nat-
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