

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

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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.

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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.¹ 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,² and interferon γ (IFN- γ), which has a number of immunostimulatory and parasitistatic effects.³ In addition, *T gondii* specific antibodies have also been implicated in the control of the acute phase of the infection.⁴ The mechanisms of control of the chronic phase of the infection, however, remain poorly understood, though

IFN- γ is thought to inhibit the spread of the parasite in the brain.⁵

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,⁶⁻⁹ 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,^{10,11} and stage specific antigens exist.^{12,13} Furthermore, western blotting studies with human serum have shown that relatively few cyst antigens are recognised.^{14,15} 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),¹⁶ 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 5×10^6 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.*¹⁷

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 $11\,000 \times g$ for 60 minutes at 4°C and the protein concentration of the antigen preparation determined using the Bradford protein assay.¹⁸

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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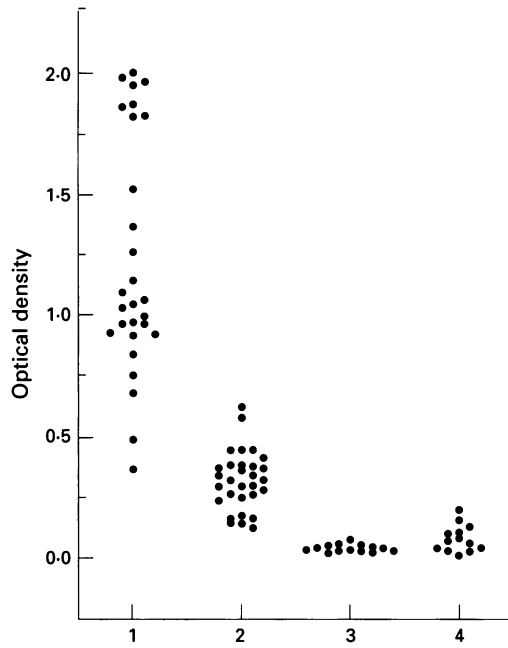


Figure 1 Serological recognition of tachyzoite (groups 1 and 3) and cyst (groups 2 and 4) antigens of *T gondii* by antibodies in serum samples taken from patients with toxoplasmosis (groups 1 and 2) and from uninfected subjects (groups 3 and 4). Individual optical density values are plotted from the results of IgG FAST-ELISA.

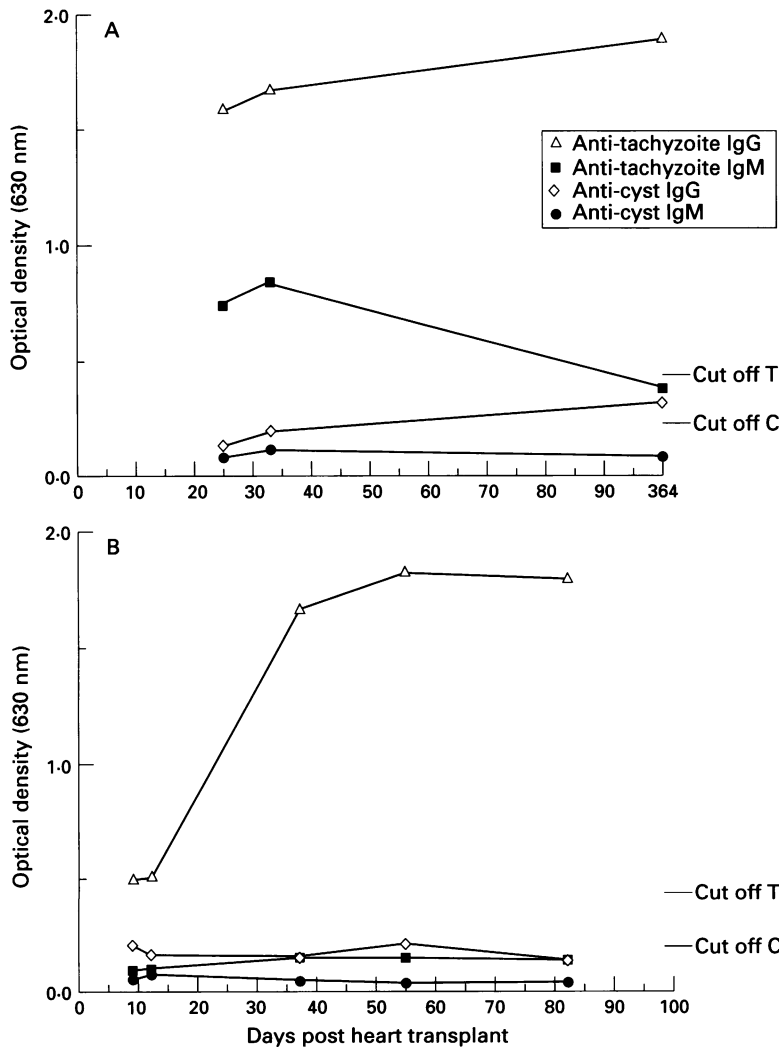


Figure 2 Human IgG and IgM antibody responses to tachyzoite and cyst antigens of *T gondii* in FAST-ELISA. Sequential serum samples were taken from two heart transplant recipients (A and B) with *T gondii* reactivation.

dye test, haemagglutination assay and IgM ELISA. 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 ethylenediamine-tetraacetic 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-

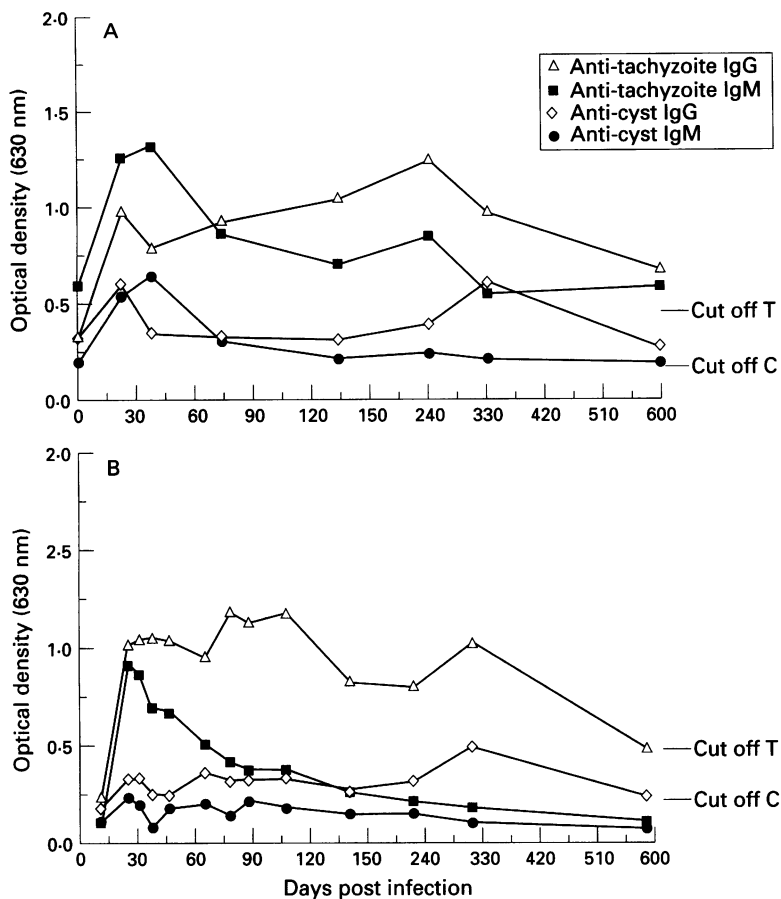


Figure 3 Human IgG and IgM antibody responses to tachyzoite and cyst antigens of *T. gondii* in FAST-ELISA. Sequential serum samples were taken from two subjects (A and B) accidentally infected with the laboratory RH strain.

cognition was low throughout the period of study (fig 2). These two patients experienced reactivation of *T. gondii* infection. In one anti-tachyzoite IgM and IgG titres were elevated at the time of diagnosis (fig 2A). Twelve months later, the IgM response had fallen but IgG remained high, recognition of cyst antigen, however, was low throughout. For the second transplant recipient we plotted the increase in anti-tachyzoite IgG over the first four weeks of infection; however, cyst antigen was not recognised nor was there any IgM response (fig 2A).

The second pair of sequential serum samples came from individuals who had been accidentally infected with the laboratory strain RH, which rarely forms cysts.¹⁹ One patient was infected by eye splash (fig 3A), the second by needlestick injury (fig 3B). In these individuals the time of infection is well documented and it is possible to follow the early rise in anti-tachyzoite IgM titres, peaking around four weeks after infection and a parallel but slightly slower increase in serum IgG. The serum IgG and IgM responses to cyst antigen were once again, less consistent, lower and rather later than the anti-tachyzoite responses.

Discussion

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

ural infection are quantitatively lower than to tachyzoite antigens.^{14,15,18} These studies suggested that bradyzoites possess fewer antigens¹³ and that the response to these is relatively low and occurs late in infection.¹⁵ A number of factors may contribute to this phenomenon. Makioka *et al.*¹⁴ suggested that the low exposure of the host to bradyzoite antigens resulted in reduced antigen recognition, while Woodison *et al.*¹⁵ postulated that cyst antigens may be inherently less immunogenic or that there may be tolerance to these antigens. In addition, the position of the tissue cysts within the central nervous and muscle tissues may lead to difficulties in the processing and presentation of cyst antigens.

Analysis of sequential serum samples showed that the individual serological IgG response to tachyzoites was rapid and strong, and remained at a high level for over 12 months after infection. In common with other authors,^{20,21} we found that the IgM response was more variable. In general, IgM antibody rose to peak levels three to five weeks after infection, then declined to baseline over time. In one transplant recipient, however, there was no IgM response. This is in agreement with earlier observations that not all heart transplant recipients with reactivation of *T. gondii* infection produce specific IgM.²²

The serological response to cyst antigen has not been monitored previously but was very low both in random serum samples and in the smaller group of patients involved in the sequential study. Only one patient had a detectable IgM titre against cyst antigen (fig 3A). This closely mirrored the anti-tachyzoite response and may reflect shared antigens, although previous western blotting studies have shown the IgM response to be predominantly directed against a small subset of tachyzoite antigens.^{7,22} The sequential IgG titre against cysts, however, was very different in profile from the anti-tachyzoite IgG, rising slowly above cut off values and reaching its highest level one year after infection.

This study highlights the strong immune response provoked by the tachyzoite stage of *T. gondii* and implies that it is this stage which predominates, both in acute primary infection and during recrudescence. In strict contrast there is only minimal recognition of the cystic stage which suggests that the cyst is able to evade the immune response and hence to persist within the patient.

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