Sequential reactivity of serum against cyst antigens in *Toxoplasma* infection

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Abstract

Aims—To compare the recognition of *Toxoplasma gondii* tachyzoites and cysts by sera, from 10 patients.

Methods—Recognition of antigens from purified tachyzoites (RH strain) and bradyzoites (18691 strain) was compared using western immunoblotting. Sequential serum samples from 10 patients and one laboratory acquired RH infection were used.

Results—Recognition of cyst antigens was relatively low and occurred late in infection. The two stages were antigenically distinct with only a few shared bands.

Conclusion—Immunological recognition of the cystic stage of *T gondii* is low. This implies that either cysts are poorly immunogenic or that cyst antigen is not available for processing and presentation.

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Toxoplasma gondii is a common protozoan parasite affecting man and domestic animals. After infection there is a transient proliferative phase followed by a chronic phase in which infection is maintained by dormant tissue cysts (bradyzoites). In normal adults the disease is self-limiting, but in immunosuppressed subjects recrudescence of the proliferating forms may lead to serious or fatal toxoplasmosis.¹ With the advent of AIDS, the incidence of cerebral toxoplasmosis has increased and has now become a major concern. Navia *et al* estimate that 30% of HIV infected patients with positive *Toxoplasma* serology will develop cerebral toxoplasmosis.²

Verhofstede, Van Gelder, and Rabaey followed the evolution of the IgG antibody response to tachyzoite antigens in sequential sera of acutely infected patients.³ The profile of antigen recognition changed during infection and they claimed that this could be used as a marker of acute or chronic disease. In AIDS associated *Toxoplasma* encephalitis, however, Weiss *et al* could find no consistent pattern of recognition.⁴

A major omission from these studies was the failure to examine immune recognition of bradyzoite antigens which are most relevant in chronic disease. Immune surveillance of cystic stages may be crucial to maintenance of these parasites and prevention of recrudescence.

In the current study we examined the sequential development of antibodies to bradyzoite and tachyzoite antigens.

Methods

The cystic strain of *Toxoplasma gondii* used in the study was a recent human isolate 18691. The strain was maintained by repeated monthly passage in adult female outbred TUCKS No 1 mice, and infection was instigated through intraperitoneal injection of 200 cysts. For biochemical analysis, cysts were harvested from infected brain tissue. Mice were drained of blood and brains were pooled, homogenised by repeated passage through a 20 gauge needle, and purified on 45% Percoll gradients according to the method of Cornelissen, Overdulve, and Hoenderboom.⁵ Cysts recovered from the gradients were washed in phosphate buffered saline (PBS), counted, and stored at -70°C . Tachyzoites were obtained from the peritoneal fluid of adult female mice infected three days earlier with the RH strain of *T gondii*. Parasites were harvested in RPMI 1640 medium (Gibco, Scotland), purified on a cellulose CF 11 (Whatman) column, counted and stored at -70°C .

Ten sets of sequential serum samples were obtained from infected patients. Clinical symptoms ranged from general malaise to lymphadenopathy and retinochoroiditis. Samples were taken throughout infection at time points of up to one year after infection, with dye test titres ranging from 8 to 16 000 (equivalent to 2–4000 IU/ml⁶). Serum samples from a laboratory acquired RH strain infection were taken at intervals between days 4 and 314 after infection, with dye test titres ranging from 8 to 8192.

Samples of 10 000 cysts and 5×10^5 tachyzoites were freeze thawed three times, heated to 95°C for 4 minutes in non-reducing buffer, and electrophoresed on 12% discontinuous polyacrylamide gels.⁷ After separation by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) proteins were electrophoretically transferred to nitrocellulose filters at 210 mA for 4 hours.⁸ Filters were then washed in PBS containing 0.05% v/v Tween for 30 minutes, dried, and cut into strips. All strips were incubated at 28°C for 45 minutes in antisera (1 in 25 dilution in PBS 0.05% Tween), then in alkaline phosphatase conjugated anti-mouse IgG (Sigma). Antigens were shown by developing strips using NBT/BCIP (Sigma) as a substrate.

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Figure 1 Western blot analysis of *Toxoplasma* tachyzoites (RH strain) and cysts (18691) strain probed with sequential sera from 10 infected patients (A–J). The first strip of each pair is tachyzoites and the second, cysts. The dye test titre at each time point is indicated.

Results

Figure 1 shows the pattern of recognition of tachyzoite and cyst antigens with sequential sera from a panel of 10 infected subjects. Overall, there was considerable heterogeneity in antigen recognition by serum samples from different patients. With cysts, prominent antigens of Mr 69 and 17×10^3 were observed frequently during late stage infection. Other minor antigens were observed at Mr 63–58, 43, 42, 40, 33, 28.5, 25 and 23×10^3 . Many of these antigens were apparent six weeks after infection. The first cyst antigen to appear was the shared tachyzoite/bradyzoite Mr 69×10^3 protein, P69, which could be detected as early as 20 days after infection. There was increased recognition of cyst

antigens with time as shown by an increase in both the intensity and number of bands.

By contrast, the intensity of antigen recognition in tachyzoites was very high, although recognition of some antigens decreased as infection progressed. A clear example of this was the reduced recognition of the dominant Mr 42×10^3 tachyzoite antigen (cases B, F, G and J). The profile of tachyzoite antigens recognised is typical, with the earliest response being to the Mr 42 and 30×10^3 antigens. P30 could still be detected one year after infection.

Figure 2 shows the pattern of recognition of tachyzoites and cysts by sequential sera from an individual with a laboratory acquired infection (RH strain) in which cysts are rarely formed. Overall, very few cyst antigens were recognised. In contrast, the response to tachyzoites was strong and the major surface antigens of Mr 42 and 30×10^3 were, typically, the first to be recognised at 25 days after infection. With time, a complex of antigens were seen around P30, and at 70 days after infection, six antigens of Mr 33, 32, 31, 30, 29 and 28×10^3 were observed. P30 could still be detected at 220 days after infection.

Figure 2 Western blot analysis of *Toxoplasma* tachyzoites (RH strain) and cysts (18691 strain) probed with sequential sera from a laboratory acquired RH infection. Dye test titres are indicated.

Discussion

The patient response to cyst antigens during natural infection varied greatly compared with the response against the proliferative tachyzoite. Two aspects should be emphasised. First, the antibody response to cysts was quantitatively lower than to tachyzoites. Several factors could have contributed to this poor response: cyst antigens may be inherently less immunogenic; antigen load may be lower; processing and presentation of cyst antigens, predominantly located in brain and muscle tissue, may be inefficient or indeed

there may be tolerance to these antigens. Second, the antigens themselves are different. Stage specific antigens have already been noted using hyperimmune serum in cross-recognition experiments.⁹⁻¹¹ The antigens recognised by human sera in this study conform to the (Mr 67, 28 and 26×10^3) antigens described before.¹²

As shown by patient sera, some antigens are unique to bradyzoites, some are shared (P69 and P42), and some tachyzoite antigens are absent from cysts. The major tachyzoite surface protein P30 was not observed on cysts either by immune sera or specific anti-P30 monoclonal antibodies (C1E3, 1E5, data not shown). Recognition of cyst antigens seems to relate to the formation of cysts. Cysts are strongly recognised by late sera, implying that once tachyzoites have encysted in brain and muscle tissue, antigens specific to the cyst are recognised. The RH strain rarely produces cysts and only the Mr 69×10^3 shared antigen was detected by this sera.

Sequential recognition of tachyzoite antigens in this study conforms to patterns reported before.^{3 13 14} The earlier IgG response is directed against molecules of Mr 42 and 30×10^3 which may represent major surface proteins. Many other antigens are recognised later, in particular around the Mr 60 and 30×10^3 regions.

The Mr 69 and 17×10^3 antigens seem to be the most relevant to the chronic stage and are recognised by most sera. The Mr 17×10^3 antigen was particularly prominent late in infection and is expressed only in cysts. This antigen may be useful in the diagnosis of chronic infection. The Mr 69×10^3 antigen is strongly immunogenic in both tachyzoites and cysts and shows no strain-dependent variation.¹² This antigen may also

be of diagnostic relevance. We are currently isolating the protein for sequence analysis.

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