SHORT COMMUNICATION

DETERMINATION OF DIAGNOSTIC VALUE OF *Toxoplasma gondii* RECOMBINANT SURFACE ANTIGEN (SAG1, P30) IN MOUSE EXPERIMENTAL MODEL

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SUMMARY

The aim of this study was to test the potential diagnostic usefulness of recombinant *Toxoplasma gondii* SAG1 antigen and excretory-secretory antigen (ESA), with respect to toxoplasmosis detection and infection phase distinction in laboratory mouse by determining specific serum IgM and IgG antibodies with the use of indirect ELISA technique. The highest titre at the beginning of infection was demonstrated by immunoglobulin M while the highest titre at the end of the infection was displayed by immunoglobulin G. In contrast, sera of chronically infected mice, positive IgG titre was detected on day 14 p.i. for ESA or day seven p.i. with rSAG1 and increased thereafter until day 70 p.i. after which the titre stabilized. IgA antibody also showed similar kinetics as IgG. Potentially rSAG1 may be a suitable diagnostic antigen than ESA in the diagnosis of acute and chronic toxoplasmosis.

Keywords: toxoplasmosis, diagnosis, recombinant, surface antigen

INTRODUCTION

Toxoplasma gondii is a ubiquitous protozoan parasite that is estimated to infect one-third of the world's human population. It can infect many species of warm-blooded animals and is a significant zoonotic and veterinary pathogen. It has been 100 years since *T. gondii* was initially described in the tissues of C*tenodactylus gundi*, a North African rodent (Nicole and Manceux, 1908). Splendore, in Brazil, reported on the identification of this organism in the tissues of a rabbit. (Splendore, 1995).

Consumption of raw meat containing tissue cysts of *T. gondii* is an important route for the infection in humans. Tissue cysts are mostly found in pigs, sheep and goats but less often in poultry, horses and cattle. (Weiss *et al*., 2009; Tenter *et al*., 2000).

The immunodominant surface antigen of *T. gondii*, surface antigen 1 (SAG1, previously named P30) is supplied with six histidyl residues in the N-terminal end. The construction can be produced in large amounts, is easy to purify, and is recognised by anti-SAG1 monoclonal antibodies, *Toxoplasma*-specific acute phase immunoglobulin M (IgM) antibodies and chronic-phase IgG antibodies. Native mature SAG1 protein is presumed to be post-translationally modified by removal of the signal sequence and the C terminus, the latter upon addition of the GPI anchor (Harning *et al*., 1996; Nagel and Boothroyd, 1989). SAG1 antigen is expressed on the surface of intra- and extra-cellular tachyzoites (Santoro *et al*., 1986).

Most serological tests require native antigens of tachyzoites harvested from mice or cell cultures. Since constant quality and specificity of these antigens are not satisfactory, the use of recombinant antigens could abolish these disadvantages, lower production and purification costs and enable selection of antigens appropriate for the discrimination of toxoplasmosis phases. Several literature data revealed the potential usefulness of recombinant antigens for toxoplasmosis diagnostics (Aubert *et al*., 2000; Buffolano *et al*., 2005; Jacobs *et al*., 1999; Pietkiewicz *et al*., 2004) and even reflect attempts to employ them as tools for *T. gondii* invasion phase differentiation, however with discordant results (Ferrandiz *et al*., 2004; Nigro *et al*., 2003; Pfrepper *et al*., 2005)

MATERIALS AND METHODS

Preparation of antigens

Excretory Secretory Antigen (ESA) was prepared from peritoneal washings of twenty mice were pooled and washed twice in RPMI-1640 with penicillin streptomycin (RPMI-PS). The pellet was then reconstituted in ten ml of RPMI-PS plus 10% fetal calf serum. One mililitre each was aliquoted into ten tubes and incubated at 37 °C for three hours under mild agitation. The content of the tubes were then pooled, pelleted and the supernatant (ESA) was filtered through 0.45 µm membrane filter.

The *Pichia pastoris* expression system was used to produce recombinant Surface antigen 1 (rSAG1). The purified of rSAG1 was analysed by gel electrophoresis to be at 30 kDa molecular weight (Wan Omar et al., 2010).

Indirect ELISA was performed on consecutive sera of mice acutely infected with *T. gondii*. The optimum concentration of antigens were found to be 5 µg/ml for rSAG1 and 80 µg/ml for ESA.

RESULTS AND DISCUSSION

Sera from mice acutely infected with *T. gondii* were analysed. Figures 1 and 2 show the ELISA results from the consecutive sera of mice acutely infected using ESA and rSAG1 respectively. With the use of ESA, positive titres for all the three antibody isotypes were observed from day seven p.i. Immunoglobulin M showed the highest titre at the beginning but IgG achieved the highest titreon day ten p.i. Using rSAG1, positive titres were detected earlier (day 5 p.i). Similar to figure 1, IgM titre was highest initially but IgG demonstrated the highest titre at the end of the infection.

Sera from mice chronically infected with *T. gondii* were analysed. Figures 3, 4 and 5 show the ELISA results from the consecutive sera of mice chronically infected, using ESA and rSAG1 respectively. With the use of ESA, IgG was positive from day 14 p.i., IgM from day three p.i. and IgA from day seven p.i. The maximum titres for IgG and IgA were observed at the end of the infection, while the maximum titre for IgM was seen on day 14 p.i. The use of rSAG1 as the solid phase demonstrated a high IgG titre (Figure 4) at the end of the infection i.e. 125 times greater than the cut off point. IgM was positive from day three p.i. (Figure 5), whereas the initiation of positive titre for IgG and IgA was observed on day seven p.i.

The results showed that there are differences in the dynamics of the development of antibody titres throughout the course of infection between acutely and chronically infected mice. In mice acutely infected with *Toxoplasma gondii*, positive titres for the three antibody isotypes were detected on days seven p.i. and five p.i. with the use of SA and rSAG1 respectively. In mice acutely infected with *Toxoplasma gondii*, positive titres for the three antibody isotypes were detected on days seven p.i. and five p.i. with the use of ESA and rSAG1 respectively. The titres increased progressively until the end of the infection. The highest titre at the beginning of infection was demonstrated by immunoglobulin M while the highest titre at the end of the infection was displayed by immunoglobulin G. In contrast, sera of chronically infected mice, positive IgG titre was detected on day 14 p.i. for ESA or day seven p.i. with rSAG1 and increased thereafter until day 70 p.i. after which the titre stabilised. IgA antibody also showed similar kinetics as IgG.

If the results may be extrapolated to infection in human patients, potentially rSAG1 may be a suitable diagnostic antigen than ESA in the detection of antibodies to Toxoplasma whereby IgM is the best marker of acute infection and IgG for chronic infection. Studies are ongoing in our laboratory in utilisation of rSAG1 as vaccine candidate for protection against lethal challenge with *T. gondii*. Preliminary observations show rSAG plus alum induces protective immunity in mice and immunised mice survive longer.

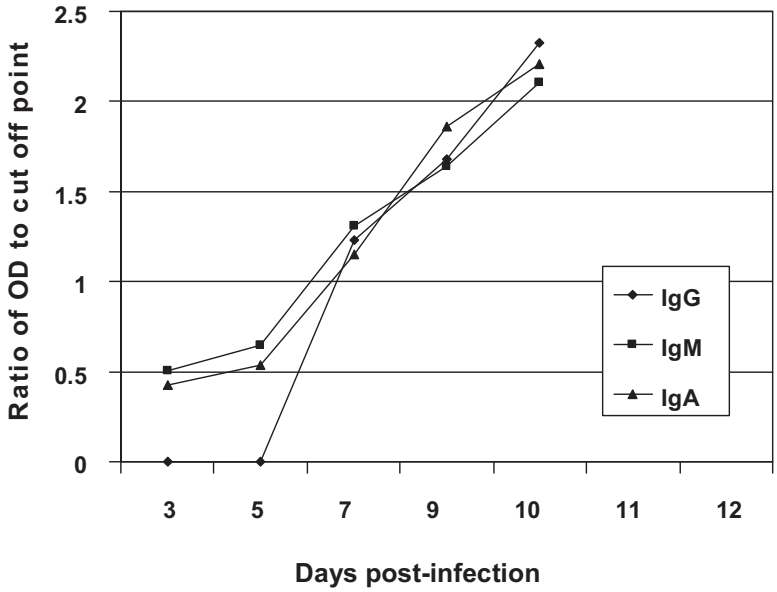

Figure 1. ELISA using ESA and consecutive sera from mice with acute *Toxoplasma* infection

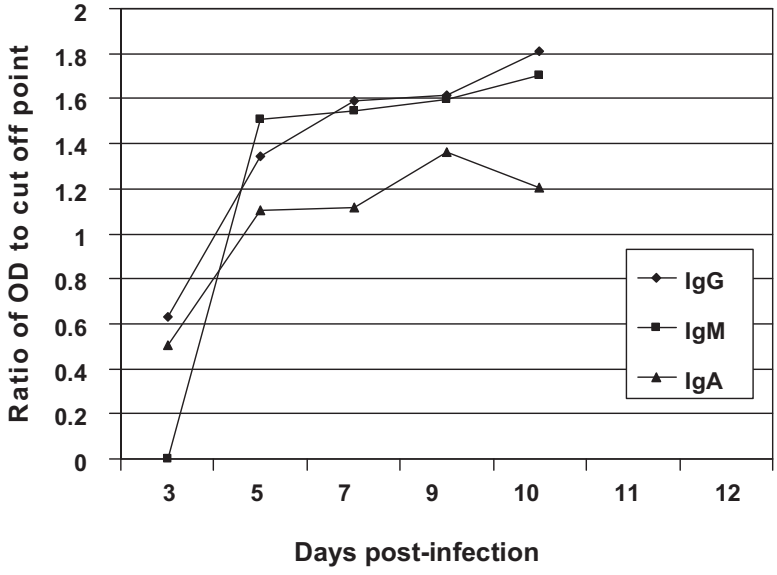

Figure 2. ELISA using rSAG1 and consecutive sera from mice with acute *Toxoplasma* infection

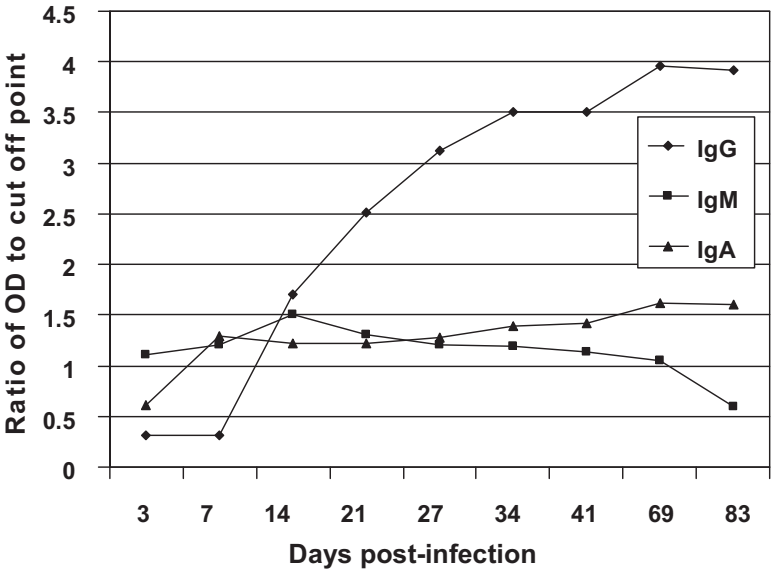

Figure 3. ELISA using ESA and consecutive sera from mice with chronic *Toxopasma* infection

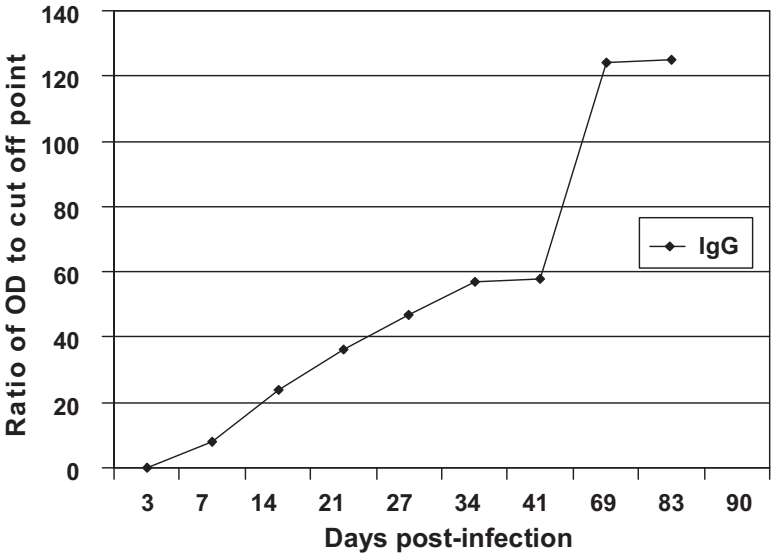

Figure 4. ELISA using rSAG1 and consecutive sera (IgG) from mice with chronic *Toxoplasma* infection

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