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Research Brief

Evaluation of the antigenic value of recombinant *Toxoplasma gondii* HSP20 to detect specific immunoglobulin G antibodies in *Toxoplasma* infected humans

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ABSTRACT

Recombinant *Toxoplasma gondii* small heat shock protein HSP20, surface antigen SAG1 and dense granule GRA7 were analyzed by IgG-ELISA with serum samples of *Toxoplasma* infected humans grouped as I (IgG+, IgM+), II (IgG+, IgM−) and III (IgG−, IgM−). rHSP20 reacted against 80% and 62.5% of serum samples from groups I and II, respectively. rSAG1 was recognized by 85% of the samples from group I and 70.8% from group II, whereas rGRA7 was recognized by 85% and 66.6% of the serum samples from groups I and II, respectively. When a combination of two or three recombinant antigens was used, the sensitivity values improved to 85–95% for group I and 87.5–91.7% for group II. All combinations tested produced similar reactivity profiles. None of the recombinant proteins reacted against group III serum samples. In conclusion, we demonstrated that *T. gondii* HSP20 elicits an important B-cell response during human infection, and could be suitable for the development of serodiagnosis tools.

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1. Introduction

The protozoan parasite *Toxoplasma gondii* is an important human and veterinary pathogen (Tenter et al., 2000). Because of the late development of the cellular immune response during fetal maturation, *T. gondii* has long been associated with congenital birth defects (Wong and Remington, 1994) and considered a cause of life-threatening opportunistic diseases in immunocompromised individuals as well (Luft and Remington, 1992).

During infection, this parasite elicits a strong humoral and cellular response which protects the host against re-infection (Denkers and Gazzinelli, 1998). Consequently, the detection of specific immunoglobulin G (IgG) antibodies along with absence of the acute-phase markers IgM and IgA allow the diagnosis of the chronic (Ch) stage of infection or a past exposure to the parasite, whereas detection of IgM and IgA suggests an active or recently acquired (RA) infection (Wong and Remington, 1994). However, since IgM antibodies may remain detectable for more than 1 year after initial infection, their detection cannot be used as a marker of acute infection (Wong and Remington, 1994).

Most serological tests for *Toxoplasma* require the preparation of parasite antigens from tachyzoites harvested from mice or cell culture systems. The use of recombinant antigens allows to significantly reduce production costs. In addition, the particular diagnostic value of each antigen can be easily studied and more than one defined antigen can be used to facilitate the standardization of diagnostic systems. In an effort to discern the parasite's antigenic mosaic, several authors analyzed the value of different recombinant antigens (Aubert et al., 2000; Li et al., 2000; Nigro et al., 2003; Pietkiewicz et al., 2004; Beghetto et al., 2006; Holec et al., 2008; Holec-Gasior et al., 2009). These investigations showed that several antigens elicit a humoral response during parasite infection in humans, but with different reactivity in serological assays. Interestingly, some of these antigens are also attractive vaccine candidates (Jongert et al., 2009). Among these antigens, dense granule antigen 7 (GRA7) detected IgG antibodies present in the early phase of infection (Jacobs et al., 1999; Li et al., 2000; Nigro et al., 2003; Pietkiewicz et al., 2004). Another antigen, the surface antigen 1 (SAG1) has been proven to be a good candidate for diagnosis (Harning et al., 1996; Wu et al., 2009), but difficult to express as soluble protein in bacteria (Makioka and Kobayashi, 1991; Chen et al., 2001; Nigro et al., 2003; Meek et al., 2003).

Recently we identified five *T. gondii* small heat shock proteins (de Miguel et al., 2005). One of them, identified as HSP20, is an inner membrane complex associated chaperone (de Miguel et al., 2008). Interestingly, antibodies against *Babesia* HSP20 block parasite growth (Montero et al., 2008). Here, we decided to analyze the reactivity of a recombinant HSP20 protein (rHSP20) with serum samples from pregnant women infected with *Toxoplasma*. Since recombinant proteins SAG1 and GRA7 (rSAG1 and rGRA7) have

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been shown to be valuable antigens for human serodiagnosis (Harning et al., 1996; Aubert et al., 2000; Nigro et al., 2003), we compared the data value of rHSP20 with those obtained with rSAG1 and rGRA7. Finally, we analyzed different combinations of these proteins to determine whether a specific combination can improve the IgG detection in human serum samples from infected individuals.

2. Materials and methods

2.1. Cloning of recombinant proteins

The DNA region encoding the aminoacid residues 77–322 (Harning et al., 1996) of SAG1 (GenBank accession No. AF132217) was amplified by PCR from the recombinant amplicon pZPVXSAG1 (Clemente et al., 2005). The following primers were used: sense 5′ gaattcttactcttaagtgtcctaagacagctcttaca and antisense 5′ gatatctcacatagcaaagatggaaacatgagaagctgt. EcoRI and EcoRV sites (underlined sequences) were included in the sense and antisense primers respectively. The *sag1* DNA fragment was cloned into the prokaryotic expression vector pRSET B (Invitrogen Life Technology), downstream and in frame with a sequence that encodes an N-terminal 6xHis-tag. *Escherichia coli* Rosetta(DE3)pLysS (Novagen) cells were transformed with the expression plasmid.

The cloning procedure and features of rGRA7 and rHSP20 have already been described (Nigro et al., 2003; de Miguel et al., 2005). Briefly, gra7 was cloned into pQE (Qiagen) and expressed in M15 cells (Qiagen), whereas HSP20 was cloned into pRSET (Invitrogen Life Technologies) and expressed into BL21(DE3)pLysS bacteria (Novagen).

2.2. Expression and purification of recombinant proteins

Transformed *E. coli* were grown in LB broth with ampicillin (100 μg/ml) whereas BL21 and Rosetta derived bacteria (rHSP20, rSAG1) were grown in LB broth with ampicillin (100 μg/ml) and chloramphenicol (32 μg/ml). For protein expression, cultures were grown to OD600 = 0.6, and induced by the addition of isopropyl thio-β-D-galactoside (1–2 mM). Bacteria expressing rGRA7 and rHSP20 were lysed with lysis buffer pH8 (300 mM NaCl, 50 mM $NaH_2PO_4{\cdot}H_2O$, 10 mM imidazole). Lysates were sonicated, centrifuged at 10,000 × *g* for 20 min at 4 °C and the supernatants were collected and directly loaded onto a Ni–NTA column previously equilibrated with lysis buffer. The column was washed four times with washing buffer (300 mM NaCl, 50 mM $NaH_2PO_4{\cdot}H_2O$, 30 mM imidazole). Bound recombinant proteins were eluted with elution buffer (300 mM NaCl, 50 mM $NaH_2PO_4{\cdot}H_2O$, 250 mM imidazole). rSAG1 was purified similarly as described above, except that the transformed cells were treated with urea lysing buffer pH 8 (100 mM $NaH_2PO_4{\cdot}H_2O$, 10 mM Tris–HCl, 8 M urea), the column was washed with washing buffer containing urea (100 mM $NaH_2PO_4{\cdot}H_2O$, 10 mM Tris–HCl, 8 M urea, pH 6.3) and rTgSAG1 eluted with 100 mM $NaH_2PO_4{\cdot}H_2O$, 10 mM Tris–HCl, 8 M urea, pH 4.5. The purity of the recombinant proteins was confirmed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. Finally, protein concentration was determined by the Bradford's method (Bradford, 1976).

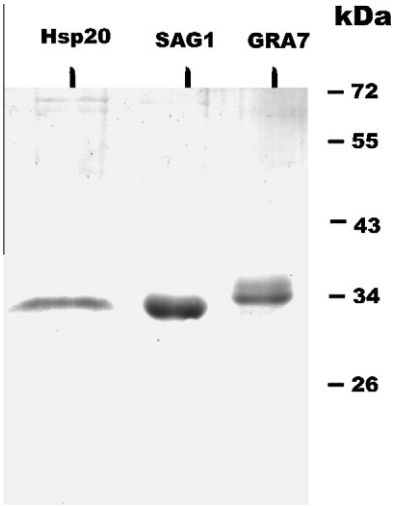

Fig. 1. Expression of *T. gondii* recombinant antigens. Recombinant proteins were expressed fused to a 6-His tag at the N-terminus in *E. coli*, purified by Ni–NTA resin, run onto a SDS–PAGE and stained with Coomassie brilliant blue. On the right side, molecular weight markers are expressed in kDa.

2.3. Serum samples

All sera used in this study were from pregnant women. Serum samples were obtained during routine serologic screening of preg-

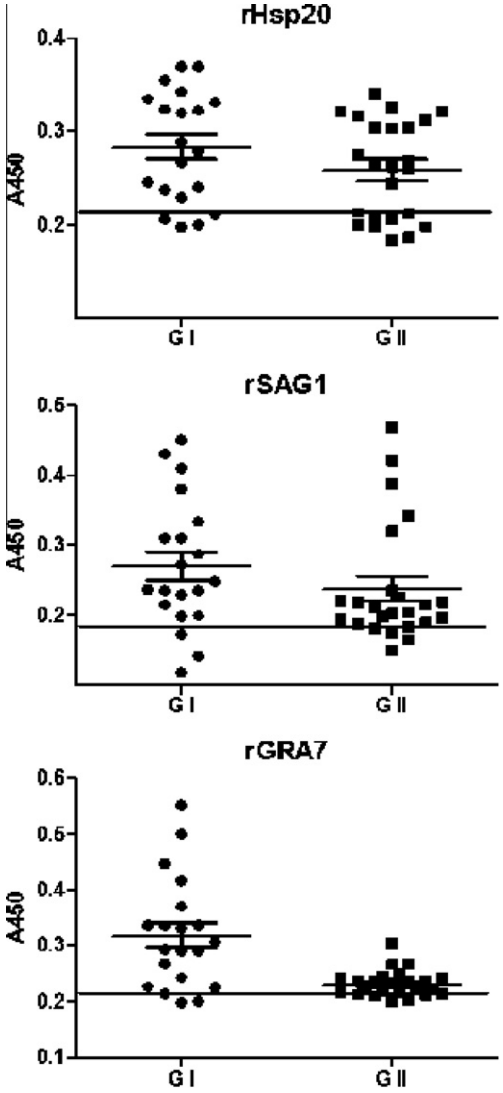

Fig. 2. Immunoreactivity of *T. gondii* recombinant antigens. ELISA microplates were sensitized with rSAG1, rGRA7, rHSP20 and incubated with sera from *Toxoplasma* infected pregnant women from G I and G II. Optical density was measured at 450 nm (A450). Cut-off value (horizontal line) was determined as the mean of the 25 sera from seronegative individuals plus three standard deviations. Positives were considered as such when A450 values were higher than cut-off.

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