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Use of SAG2A recombinant *Toxoplasma gondii* surface antigen as a diagnostic marker for human acute toxoplasmosis: analysis of titers and avidity of IgG and IgG1 antibodies

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Abstract

We evaluated the reactivity of IgG and IgG1 antibodies by immunoassays in sera from patients with acute and chronic phases of toxoplasmosis against 2 recombinant antigens, SAG2A (full molecule) and SAG2AΔ (truncated molecule from the epitope recognized by A4D12 monoclonal antibody [mAb]), in comparison with soluble *Toxoplasma* antigen (STAg). Results demonstrated higher IgG reactivity in acute sera with both STAg and SAG2A than in chronic phase sera, and this difference was more evident for IgG1 antibodies to SAG2A. Low reactivity to SAG2AΔ was found in sera from both phases. ELISA-IgG-SAG2A showed high sensitivity (95%) and specificity (100%). ELISA-IgG1-SAG2A sensitivity was significantly higher (90%) for acute than for chronic (67%) phases. ELISA-IgG avidity using STAg demonstrated high performance for characterizing sera with high avidity (>60%), whereas the ELISA-IgG1 avidity-SAG2A immunoassay was the best to define chronic phase infection. It can be concluded that SAG2A is an antigen that may be used as a diagnostic tool to characterize the acute phase *Toxoplasma gondii* infection. Also, the epitope recognized by A4D12 mAb may be critical for the recognition of this molecule.
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1. Introduction

Toxoplasma gondii, a member of the phylum Apicomplexa, is an obligate intracellular and ubiquitous protozoan parasite that infects a broad range of hosts, including humans and domestic animals (Dubey et al., 1998). In immunocompetent individuals, acute infection commonly resolves without treatment, but the host remains chronically infected lifelong. In contrast, immunocompromised individuals, such as those infected with the human immunodeficiency virus or receiving immunosuppressive therapy, are at risk for life-threatening disease that develops after reactivation of quiescent brain cysts. In addition, *T. gondii* infection causes significant morbidity and mortality in congenitally infected subjects (Di Cristina et al., 2004; Golkar et al., 2007).

The parasite exhibits 3 morphologically distinct infectious stages: tachyzoites, bradyzoites (in tissue cysts), and sporozoites (in oocysts) (Dubey et al., 1998). The surface of *T. gondii* tachyzoites and bradyzoites is covered with glycosylphosphatidylinositol-anchored antigens (Nagel and Boothroyd, 1989; Tomavo et al., 1989), most of which are members of the surface antigen 1 (SAG1) or SAG2 families (Boothroyd et al., 1998; Lekutis et al., 2000; Manger et al., 1998). These molecules appear to play a role in host cell invasion, immune modulation, and/or virulence attenuation, although they may also provide protection needed by the parasite to survive in the environment, and your native or recombinant shapes are used for the serologic diagnosis of toxoplasmosis (Harning et al., 1996;

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Manger et al., 1998; Parmley et al., 1992). A 22-kDa protein, SAG2A, was identified using antigen-specific monoclonal antibody (mAb) to screen expression libraries (Prince et al., 1990), and it was originally described as being expressed specifically on the tachyzoite surface (Lekutis et al., 2000).

There are 2 major situations in which the diagnosis of *T. gondii* infection has medical attention: 1st, to detect the transmission of parasites via placenta from an infected mother to the fetus, and 2nd, to detect the reactivation of a chronic infection in immunocompromised patients (Holec et al., 2007). The diagnosis of toxoplasmosis by serologic methods routinely uses immunoenzymatic assays to detect the presence of various classes of anti-*Toxoplasma* immunoglobulins (IgG, IgM, and IgA) (Remington et al., 2001) and IgG avidity tests (Beghetto et al., 2003). The specificities and sensitivities of these serologic methods and the differentiation between the acute and chronic phases of toxoplasmosis depend mostly on the diagnostic antigen(s) used (Holec et al., 2007).

At present, the detection of specific antibodies based on the recognition of crude *Toxoplasma* antigens requires mass production of the parasite either from the peritoneal fluids of infected mice or from tissue cultures (Pietkiewicz et al., 2004). Several recent studies have reported the use of recombinant proteins for the serologic diagnosis of *T. gondii* infection (Beghetto et al., 2003, 2006; Buffolano et al., 2004; Di Cristina et al., 2004; Golkar et al., 2007; Holec et al., 2007; Nigro et al., 2003; Pietkiewicz et al., 2004) with an advantage for reducing test costs due to the lower costs of production and purification of recombinant antigens (Pietkiewicz et al., 2004). Furthermore, properly selected recombinant antigenic proteins as specific molecular markers should detect all serologically positive individuals as well as differentiate between acute and chronic infections. Nevertheless, none of the assays based on recombinant antigens have demonstrated a large potential to replace the conventional IgG- and IgM-based tests using native antigens, indicating that additional studies are needed to characterize new diagnostic markers that could be available for clinical purposes (Beghetto et al., 2006).

In the present study, we investigated the titers and avidity of IgG and IgG1 antibodies against SAG2A recombinant antigen in comparison with soluble *Toxoplasma* antigen (STAg) in sera from patients with acute and chronic phases of toxoplasmosis and evaluated the usefulness of the SAG2A recombinant antigen in immunoassays as a potential diagnostic marker for human acute toxoplasmosis.

2. Materials and methods

2.1. Patients and serum samples

A total of 83 human serum samples was studied and divided into 3 groups according to the following criteria: I) 30 serum samples from patients with recent infection as defined by presenting positive IgG, IgM, and IgA antibodies to *T. gondii* in conventional serologic assays (indirect ELISA-IgG, capture ELISA-IgM, and capture ELISA-IgA, respectively). Among these samples, 20 of them came from patients with primary infection during pregnancy or with congenital infection at birth, whereas the remaining 10 samples were from cases of postnatal infection; II) 30 serum samples from patients with chronic infection as defined by presenting positive IgG, but negative IgM and IgA antibodies to *T. gondii*; and III) 23 serum samples from subjects with negative IgG, IgM, and IgA antibodies to *T. gondii* in the above mentioned serologic assays. All serum samples were obtained from patients who had been referred to the Infectious Diseases Clinic, Clinical Hospital of the Federal University of Uberlândia, Brazil, between January and December 2006. This study was approved by the ethical committee from this institution.

2.2. Soluble Toxoplasma antigen

T. gondii (RH strain) tachyzoites were maintained in Swiss mice by serial passage for 48 to 72 h (Mineo et al., 1980). Parasite suspension was treated with protease inhibitors (10 μg/mL aprotinin, 50 μg/mL leupeptin, and 1.6 mmol/L phenylmethylsulfonyl fluoride; all from Sigma Chemical, St. Louis, MO) and then submitted to freeze–thaw and sonication cycles (Scott et al., 1987). After centrifugation (10 000 × *g*, 15 min, 4 °C), the supernatant was collected, the protein content was determined (Lowry et al., 1951), and the aliquots were stored at −20 °C.

2.3. Construction of expression plasmid

The nucleotide sequence on the *T. gondii* gene encoding the SAG2A antigen was obtained from the BLASTp analysis of database. Peptide sequences were obtained from the epitope mapping based on phage display from the A4D12 mAb (Cunha-Júnior, 2005). *T. gondii* RH strain tachyzoites were used to isolate genomic DNA (Doyle and Doyle, 1990), which was used as the template for amplification of the SAG2A gene by a standard polymerase chain reaction (PCR) amplification protocol using the following primers containing *Nde*I and *Hin*dIII recognition sequences: 5′ CAAGTTCGCTCATATGTCCACCACCG-3′ and 5′-GACTTTCGCAAAGCTTCTCCGAAAG-3′ for full SAG2A, generating a 607-bp fragment; for amplification of SAG2A truncated without the carboxy-terminal region that contains the epitope recognized by A4D12 mAb, the same upstream primer in combination with the following downstream primer was used: 5′-AGAACCATCAAAGCTTCGACCAGCG-3′. These primers were designed from GenBank (accession number AAO72427). This strategy resulted in a fragment around 349 bp, which encoded a SAG2A protein deleted from the amino acid 135 up to carboxy-terminal region (SAG2AΔ). The PCR products were digested by restriction enzymes and inserted into the *Nde*I/*Hin*dIII sites of the pET28a vector used for

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