JOURNAL OF PROTEOMICS 77 (2012) 423–432

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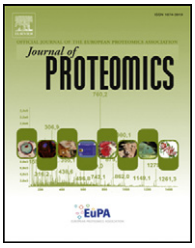

Identification of antigenic proteins of *Toxoplasma gondii* RH strain recognized by human immunoglobulin G using immunoproteomics

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ARTICLE INFO

Article history:
Received 20 June 2012
Accepted 16 September 2012
Available online 28 September 2012

Keywords:
Toxoplasma gondii
2-DE
Immunoblot
Mass spectrometry
Immunoproteomics

ABSTRACT

Toxoplasma gondii, a ubiquitous intracellular protozoan, infects one third of the world human population. It is of great medical significance, especially for pregnant women and immune-compromised patients. Accurate and early detection of *T. gondii* infection is crucial in the management of this disease. To obtain potential diagnostic markers, immunoproteomics was employed to identify immunodominant proteins separated by 2-D immunobloting and probed with sera collected from *Toxoplasma*-positive pregnant women. MALDI-TOF MS and MS/MS analyses identified a total of 18 immunoreactive proteins that were recognized by *Toxoplasma*-positive sera, whereas none was reactive with the negative-control sera from healthy, *Toxoplasma*-negative volunteers. Pregnant women showed a diverse immunoreactivity pattern with each serum recognizing one to eight identified tachyzoite proteins. The identified proteins were localized in the membrane, cytoplasm and specific organelles of *T. gondii*, and are involved in host cell invasion, metabolism and cell structure. Among these 18 proteins, actin, catalase, GAPDH, and three hypothetical proteins had a broad reactivity with *Toxoplasma*-positive sera, *indicating their potential as diagnostic markers for toxoplasmosis*. Each of several combinations of the identified proteins offered 100% detection of *Toxoplasma* infections of all 28 *Toxoplasma*-positive women. The study findings suggest that *Toxoplasma* tachyzoites are highly immunogenic and highlights the heterogeneity of host responses to *Toxoplasma* infection and the importance of using combinations of immunogens as diagnostic antigens. *The findings have significant implications to the development of diagnostic reagents with high sensitivity and specificity*.

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1874-3919/$ – see front matter.
http://dx.doi.org/10.1016/j.jprot.2012.09.018

1. Introduction

Toxoplasma gondii is a very common apicomplexan parasite of almost all warm-blooded animal species with a ubiquitous worldwide distribution. It has become one of the most important zoonoses owing to its exceptionally broad host ranges, high prevalence rate in humans and animals and its serious consequences in pregnant women and immune-compromised patients [1]. Despite its great medical importance, there still exist deficiencies in both the prevention and diagnosis of this disease. Live attenuated vaccines which can stimulate protective cell-mediated Th1 response might be a promising vaccine to prevent *Toxoplasma* infection in humans. Although a commercially available live vaccine against *T. gondii* has been successfully applied to control abortion in sheep, yet no vaccine is available for human use since there is a widespread concern that it might revert to a virulent form [2]. As an alternative to the traditional live or inactivated vaccines, novel vaccines such as synthetic peptide vaccines, DNA vaccines and subunit vaccines based on immunogenic proteins represent a promising vaccine strategy for toxoplasmosis prevention.

Apart from prevention, accurate and early diagnosis of *T. gondii* infection is critical for an effective therapy in immune-compromised patients and congenitally infected children. The practical approach in the diagnosis of *Toxoplasma* infection has been so far the serological detection of specific antibodies against *T. gondii*. However, the serological assays are generally not designed to differentiate recently acquired and past infections. Currently, the detection of IgM in the patients' sera and the IgG avidity test are being used as serological diagnosis of active toxoplasmosis [3]. The fact that IgM may persist for several years after acute infection and the difficulty in determining the cut-off values for the IgG avidity test make these two tests unsatisfactory for an earlier and accurate diagnosis [4]. Furthermore, the commercial tests available today to detect antibodies rely mostly on preparations of crude parasite antigens, which is a source of false-positivity and -negativity in serodiagnostic tests [5].

It is imperative to identify potential diagnostic markers characterizing the risk of infection and vaccine candidates for protection. Immunoproteomics, combining conventional proteomics with serology is a powerful method to identify immunodominant antigens with diagnostic and protective values. The identification of human immunodominant B-cell epitopes of *T. gondii* antigens can help discover antigens involved in the specific B-cell response and to be utilized for immunoassays to detect anti-*Toxoplasma* antibodies [5]. Previous researches on *T. gondii* using immunoproteomics identified proteins with either B-cell epitopes [6] or T-cell epitopes [7]. These antigenic proteins are associated with physiological functions of the parasite [8]. Among these antigens, some proteins such as dense granule antigens (GRAs), rhoptry antigens (ROPs), and microneme antigens (MICs) are important proteins of survival in the host and are already known antigens with good immunogenicity, and others like glutamate dehydrogenase (GDH) are enzymes and their immunogenicity need to be further evaluated [8]. GRAs are secreted by a vesicular organelle-dense granule of *T. gondii*, and participate in the modification of the parasitophorous vacuole (PV) and PV membrane for the maintenance of intracellular parasitism in almost all nucleated host cells [9]. ROPs are released by the rhoptries after attachment has been completed and at the commencement of invasion [10] and are important in invasion of host cells. MICs are released by micronemes which are small, tubular shaped organelles, and provide a connection between the host cell and the actin/myosin-based motor of the parasite [11]. However, owing to the increasing importance of *Toxoplasma* infection, more potential diagnostic markers for the accurate and early detection should be mined and heterogeneity of host immune responses be further studied.

In the present study, we applied immunoproteomics to identify potential immunogens of *T. gondii* using sera collected from pregnant women with *Toxoplasma* infection, and to study the patterns of host immune responses.

2. Materials and methods

2.1. Maintenance and purification of *T. gondii* RH tachyzoites

T. gondii RH strain was used in the present study. Tachyzoites were maintained in Vero cell monolayer, with Dulbecco Modified Eagle Medium (HyClone, USA) supplemented with 5% fetal calf serum. Infected cells were cultured at 37 °C in 5% CO_2 atmosphere for 3 to 4 days. For purification, tachyzoites were harvested by scraping the infected Vero cell monolayer into the growth medium. Parasites and host cell debris were washed in phosphate-buffered saline (PBS, pH 7.4), and the final pellet was re-suspended in PBS and passed three times through a 27-gage needle syringe. Parasites were then filtered through a 5.0 μm pore filter (Millipore, MA, USA), washed twice with 10 ml of PBS, and pelleted by centrifugation at 1000×*g* for 10 min. Purified tachyzoites were stored at −70 °C until further use.

2.2. ELISA and IFA assays for screening positive sera of pregnant women infected with *T. gondii*

ELISA and IFA tests were used to screen positive sera collected from pregnant volunteers. One thousand pregnant women were recruited for *Toxoplasma* infection detection. *Toxoplasma*-specific IgG antibodies in 1000 serum samples were tested by an ELISA kit (Haitai, China) according to the manufacturer's instruction and the results were further verified by IFA developed in the current study. Briefly, 100 μl of each serum sample diluted at 1:100 was added to the micro-ELISA plate wells in duplicates. HRP-labeled goat anti-human IgG and TMB reagents were used to detect the antigen–antibody reaction. The plates were read in a Model 680 microplate reader (Bio-Rad, USA) at 450 nm. Negative and positive control wells were included in each plate. The cut-off point of OD values of a positive sample was set to be at least two times higher than that of the negative samples at any dilution point. All sera with positive reactivity in ELISA were further tested with the IFA assay [12]. Briefly, to a suspension of 4×10^7/ml *T. gondii* RH strain tachyzoites in PBS, 40% formaldehyde was added to give a final formaldehyde concentration of 0.2% (v/v). After incubation at 4 °C overnight, aliquots of the fixed tachyzoites were stored frozen in sealed tubes until use. Five μl of the fixed whole