Identification of cross-reactive antigen of *Plasmodium falciparum* able to control *Toxoplasma gondii* infection

Zhang, H\(^1,2\), Ibrahim, H.M\(^1,3\), Fukumoto, S\(^1\), Jin, L\(^4\), Ishii, M\(^5\), Takeo, S\(^4\), Igarashi, M\(^1\), Yokoyama, N\(^1\), Xuan, X\(^1\), Kojima, N\(^5\), Tsuboi, T\(^4,6\) and Nishikawa, Y\(^1\)*.

\(^1\)National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan, \(^2\)Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, No. 518 Ziyue Road, Minhang District, Shanghai 200241, China, \(^3\)Zoology Department, Faculty of Science, Minufiya University, Shibin El Kom, Egypt, \(^4\)Cell-Free Science and Technology Research Center, Ehime University, Matsuyama, Ehime 790-8577, Japan, \(^5\)Institute of Glycoscience, Tokai University, Hiratsuka, Kanagawa 259-1292, Japan, \(^6\)Venture Business Laboratory, Ehime University, Matsuyama, Ehime 790-8577, Japan

*Corresponding author: Nishikawa, Y., E-mail: nisikawa@obihiro.ac.jp

**ABSTRACT**

Apicomplexan parasites have been shown to elicit a potent, cell-mediated immune response that can not only efficiently control the growth of the invading parasite, but also lead to nonspecific resistance to unrelated pathogens. In this study, we identified a cross-protective antigen of *Plasmodium falciparum* able to control *Toxoplasma gondii* infection.

**Keywords:** *Plasmodium falciparum*; *Toxoplasma gondii*; Sporozoite; MSP10; TRAP

Apicomplexa is a protozoan phylum comprising obligate intracellular parasites characterized by an apical complex containing organelles, such as micronemes and rhoptries (Morrisette and Sibley, 1992). *Plasmodium* and *Toxoplasma* are responsible for high rates of morbidity and mortality in humans, particularly in economically underdeveloped regions of the world. *Plasmodium* parasites alone are annually responsible for over 300 million cases of human malaria, resulting in up to 3 million deaths (Breman, 2001). Toxoplasmosis mediated by *Toxoplasma gondii* is an opportunistic infection and a major cause of morbidity and mortality amongst immunocompromised patients, particularly those infected with HIV (Luft and Remington, 1988). Co-infection with HIV and malaria is also particularly common in Africa, resulting in severe malarial anemia (Otieno et al., 2006). Apicomplexan parasites are serious problems in parts of the world without the infrastructure to control them.

Apicomplexan parasites have been shown to elicit a potent, cell-mediated immune response that can not only efficiently control the growth of the invading parasite, but also lead to nonspecific resistance to unrelated pathogens and tumors. *T. gondii* or *Trypanosoma cruzi* infection have been shown to induce resistance to murine and rat tumors (Hibbs et al., 1971; Hunter et al., 2001; Miyahara et al., 1992a,b; Oliveira et al., 2001). In addition, *T. gondii* infection conferred protection against infection with *Schistosoma mansoni* or *P. yoelii* sporozoites (Charest et al., 2000; Mahmoud et al., 1976). Moreover, immunization with heat-killed *T. gondii* conferred protection against *P. yoelii* (Haque et al., 1999). Therefore, we hypothesized that *Plasmodium* parasites will have antigens able to react with *T. gondii*. In the present study, to confirm this idea, we examined *Plasmodium falciparum*-derived antigen(s) able to stimulate cross-protective activity to control *T. gondii*.

Liver stages of *Plasmodium* parasites, sporozoites, are able to invade hepatocytes and transform
into exoerythrocytic stages. On the other hand, *T. gondii* can actively invade both phagocytic and non-phagocytic cells, but not red blood cells. The infection dynamics of *Plasmodium* sporozoite is similar to the *T. gondii*. Thus, we focused on the proteins expressed in sporozoite stage for discovering cross-reactive antigens with *T. gondii*. BLAST searches using the gene sequences of *T. gondii* (ToxoDB, http://www.toxodb.org/toxo/home.jsp) and *Plasmodium* (PlasmoDB, http://www.plasmodb.org/plasmo/home.jsp) revealed 2 sporozoite proteins of *P. falciparum* (merozoite surface protein 10 (PfMSP10, Gene ID: PFF0995c) and thrombospondin-related anonymous protein (PfTRAP, Gene ID: PF13_0201)) that were potential candidates for cross-reaction with both *T. gondii* (micronemal protein 8 (MIC8, Gene ID: 50.m00002) and MIC2 (Gene ID: 20.m00002), respectively). We also confirmed PfMSP10 expression by *P. falciparum* sporozoites (unpublished data). PfMSP10 protein, based on 3D7 strain sequence (PFF0995c), was expressed as a glutathione S-transferase (GST)-fusion recombinant protein using a wheat germ cell-free protein synthesis system. Proteins were then purified by glutathione-affinity chromatography followed by tobacco etch virus protease cleavage to remove the GST tag. PfTRAP (PF13_0201) was also expressed and affinity purified in the same manner as PfMSP10 protein. Briefly, the full-length insert of PfMSP10 was amplified by polymerase chain reaction from cDNA from the blood-stage *P. falciparum* 3D7 strain and subcloned into pEU-E01-GST(Tev) at XhoI/NcoI sites. The full-length cDNA insert of PfTRAP was also amplified, as described above. After confirmation of the nucleotide sequence, we synthesized the recombinant protein using a wheat germ cell-free protein expression system and the bilayer translation reaction method (Tsuboi et al., 2008).

In order to confirm the cross-reactivity of PfMSP10 and PfTRAP with *T. gondii*, splenocytes from *T. gondii*-primed mice were cultured with the sporozoite proteins (Fig. 1A). Antigen-specific IFN-γ production was found in the *T. gondii*-primed splenocytes cultured with 50 μg/ml MSP10 and TRAP, indicating that the *P. falciparum* sporozoite proteins, PfMSP10 and PfTRAP, were cross-reactive with *T. gondii* antigens. To assess if PfMSP10 or PfTRAP conferred protection against *T. gondii* infection, we examined the protective effects of the *P. falciparum* sporozoite proteins against *T. gondii* infection. Previous studies have indicated that the protection against *T. gondii* infection involves a Th1-type cell mediated immune mechanism. Therefore, we used a system of oligomannose-coated liposome because it is able to induce the entrapped antigen-specific Th1-type immune response. In this experiment, we used a recombinant protein of *T. gondii* apical membrane antigen 1 (TgAMA1) as a control of homogenous antigen. The antigen-immunized mice were challenged with *T. gondii* tachyzoites and monitored for a 30-day period (Fig. 1B). Mice immunized with TgAMA1 showed 58.8% protection against *T. gondii* challenge. Immunization of mice with PfMSP10 conferred 41.7% protection, while control immunization with PBS and glutathione S-transferase (GST) failed to confer protection. Moreover, PfTRAP immunization also failed to confer significant protection (18.2%).
Fig. 1. (A) IFN-γ production by splenocytes from BALB/c mice infected with *T. gondii* in the presence of PfMSP10 and PfTRAP. A single-cell suspension was prepared from spleens of mice (*n* = 4) that had survived infection with 10^6 *T. gondii* tachyzoites (PLK strain) or immunization with PBS. The cells were stimulated by adding 50 μg/ml of *T. gondii* lysate (TLA), PfMSP10 or PfTRAP. After incubation for 48 h at 37°C, the supernatants of cultures were collected and assayed for IFN-γ using enzyme-linked immunosorbent assay (ELISA) kits (Pierce, Rockford, IL). Each value represents the mean ± standard deviation of quadruplicate samples. (B) Survival rates of mice immunized with TgAMA1, PfMSP10 or PfTRAP against *T. gondii* infection. Oligomannose-coated liposomes were prepared as described previously (Nishikawa et al., 2009). Female BALB/c mice were inoculated subcutaneously with 40 nmol liposome-entrapped PfMSP10, PfTRAP, GST-fused TgAMA1 (Zhang et al., 2007) and GST (PfMSP10, PfTRAP, TgAMA1 and GST, respectively) or PBS alone (100 μl each). Booster immunizations were administered at 7 and 14 days after the first immunization. Seven days after the third immunization, the mice were challenged intraperitoneally with 10^5 *T. gondii* tachyzoites (PLK strain) and monitored for 30 days. Significant differences between treated and control (PBS) groups at 30 days after challenge were determined by χ^2^-tests (*P* < 0.01).

To investigate the protective mechanism induced by PfMSP10, sera from mice immunized with the protein were tested by enzyme-linked immunosorbent assay (Fig. 2A). Antibodies against PfMSP10 were detected, but anti-PfMSP10 antibody did not react with *T. gondii* antigens (TLA). This result indicates that anti-PfMSP10 antibody dose not contribute to the protective responses. Next, we focused on the effects of T cell responses induced by immunization with PfMSP10. Mice were treated with anti-CD4 and anti-CD8 monoclonal antibodies to deplete CD4^+^ cells and CD8^+^ cells, respectively. As shown in Fig. 2B, all PfMSP10-immunized mice died following *T. gondii* infection after treatment with anti-CD4 or anti-CD8 monoclonal antibodies. These results suggest that immunization with PfMSP10 induced partial resistance to *T. gondii* in antibody-independent and T cell-dependent manners.
Fig. 2. (A) Female BALB/c mice were inoculated subcutaneously with 40 nmol liposome-entrapped PfMSP10 and GST (PfMSP10 and GST, respectively) or PBS alone (100 μl each). Booster immunizations were administered at 7 and 14 days after the first immunization. Serum (20 μl) was obtained from mice (n = 6) via the tail vein at 7, 14, and 21 days after immunization for measurement of T. gondii-specific antibodies, by ELISA using GST, PfMSP10 and TLA as antigens (Ag), as described previously (Nishikawa et al., 2009). GST was used as the ELISA antigen for the PBS group. The ELISA result was determined by the mean optical density at 415 nm (OD415nm). Each value represents the mean ± standard deviation of 6 samples. (B) Survival rates of mice immunized with PfMSP10 and treated with anti-CD4 or anti-CD8 monoclonal antibody (mAb) against T. gondii infection. Female BALB/c mice were inoculated subcutaneously with 40 nmol liposome-entrapped PfMSP10, or PBS alone (100 μl each). Booster immunizations were administered at 7 and 14 days after the first immunization. Seven days after the third immunization, the mice were challenged intraperitoneally with 10^3 T. gondii tachyzoites (PLK strain) and monitored for 30 days. GK1.5 and 53-6.72 mAbs were used for depletion of CD4+ and CD8+ T cells, respectively (Nishikawa et al., 2001). The mice immunized with liposome-entrapped PfMSP10 were inoculated intraperitoneally with 0.5 mg of GK1.5 mAb or 53-6.72 mAb 2 days before challenge and 5 days after challenge. After treatment of mAbs, percentage of CD4+ or CD8+ T cells in the peripheral blood mononuclear cells was more than 1% (data not shown).

PfTRAP from Plasmodium sporozoites (Robson et al., 1988), the parasite form that infects the salivary glands of the mosquito vector and the liver of the mammalian host, is a candidate ligand for interaction with host cell or substrate receptors. A putative PfTRAP ortholog (MIC2) has been identified in T. gondii (Wan et al., 1997). Splenocytes from mice infected with T. gondii could react with PfTRAP to produce IFN-γ, indicating that PfTRAP may possess common T cell epitopes with T. gondii antigens, such as MIC2. Alignment analysis of PfTRAP and MIC2 identified 2 conserved domains (PfTRAP, 52 LMDCSGSIR 60 and 259 WSPCSVTCG 258, MIC2, 34 LIDSSSIG 42 and 233 WSPCSVSCG 241), which could be important for the observed cross-reactivity. Black et al. (2003) identified and characterized PfMSP10, a
third member of a family of asexual stage proteins in *P. falciparum* that are glycosylphosphatidylinositol-linked, undergo post-translational processing and contain 2 epidermal growth factor-like domains. Mass spectrometry-based evidence for expression of PlasmoDB revealed that PfMSP10 was expressed in sporozoites, in addition to trophozoites, schizonts and merozoites. The putative ortholog of PfMSP10 in *T. gondii* was MIC8. Secretion of MIC8 is induced by an increase in intracellular calcium concentration and is essential for invasion of the host cell by the parasite (Kessler *et al.*, 2008). Recent study has shown that DNA vaccine expressing MIC8 conferred partial protection against *T. gondii* infection in mice (Liu *et al.*, 2010). These observations suggested the possibility of determining the ability of PfMSP10 to induce host protective responses against *T. gondii* in an in vivo model. The results of the present study demonstrated that immunization with PfMSP10 conferred partial protection against *T. gondii* in antibody-independent and T cell-dependent manners. Comparison of the amino acid sequences of PfMSP10 and MIC8 identified one similar domain (**PfMSP10**, 438 QCRCRPNYI 446, **MIC8**, 740 QCTCNPGYV 748) while this domain was not T cell epitope. Further investigations are needed to investigate the ability of these peptide sequences to react with heterologous antigens.

The present study indicated that *Plasmodium* possesses antigens that are cross-reactive with *T. gondii*. This is the first report to demonstrate that a heterologous protein can confer protection against a heterogeneous parasite. The identification of several cross-reactive antigens for heterogeneous microbes would aid in the development of multivalent vaccines, as well as improving our understanding of parasite life cycles.

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