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Development of monoclonal antibodies against *Plasmodium falciparum* thioredoxin peroxidase 1 and its possible application for malaria diagnosis

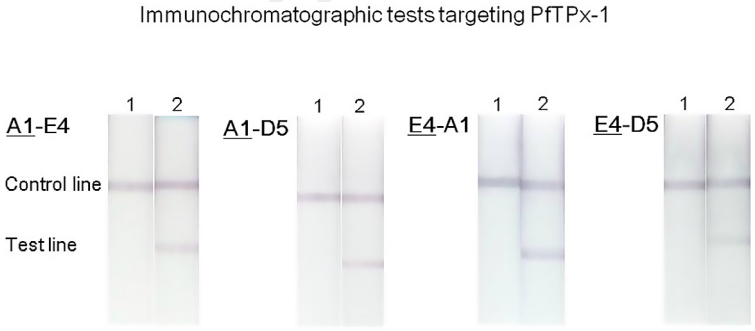
Hassan Hakimi [a,b], Yasuyuki Goto [c], Keisuke Suganuma [a], Jose Ma M. Angeles [a], Satoru Kawai [d], Noboru Inoue [a], Shin-ichiro Kawazu [a,*]

[a] National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan
[b] Department of Protozoology, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki 852-8523, Japan
[c] Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, 113-8657, Japan
[d] Laboratory of Tropical Medicine and Parasitology, Dokkyo Medical University, Tochigi Mibu 321-0293, Japan

HIGHLIGHTS

- Several monoclonal antibodies were produced against *Plasmodium falciparum* TPx-1.
- Several ICTs were developed using different combination of monoclonal antibodies.
- These tests showed positive reaction with *in vitro* culture supernatant.
- It indicates the release of TPx-1 during schizont rupture.

GRAPHICAL ABSTRACT



Monoclonal antibodies A1 or E4 was used as gold conjugate (underlined).
Monoclonal antibodies A1, E4 or D5 was used in test.
Lane 1: supernatant of non-infected culture was used as negative control.
Lane 2: *P. falciparum in vitro* culture supernatant was used.

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ABSTRACT

Rapid diagnostic tests (RDTs) have been considered as an ideal alternative for light microscopy to detect malaria parasites especially in remote areas. The development and improvement of RDTs is an area of intensive research in the last decade. To date, few parasite proteins have been targeted in RDTs which are known to have certain deficiencies and made the researchers to look for other promising candidates to address this problem. *Plasmodium falciparum* thioredoxin peroxidase 1 (PfTPx-1) is abundantly

Abbreviations: PfTPx-1, *Plasmodium falciparum* thioredoxin peroxidase 1; mAb, monoclonal antibody; ICT, immunochromatographic test; RDT, rapid diagnostic test; HRP-2, histidine rich protein 2; pLDH, plasmodial lactate dehydrogenase; Prx, peroxiredoxin; PvTPx-1, *P. vivax* TPx-1; PkTPx-1, *P. knowlesi* TPx-1; HAT, hypoxanthine-aminopterin-thymidine; ELISA, enzyme linked immunosorbent assay; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

* Corresponding author. Fax: +81 155 495643.
E-mail address: skawazu@obihiro.ac.jp (S. Kawazu).

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expressed in the cytoplasm of the parasite and well conserved across *Plasmodium* species. making this antigen a promising target for malaria diagnosis. Several monoclonal antibodies (mAbs) were produced against PfTPx-1. The binding affinities of mAbs were measured. Several immunochromatographic tests (ICTs) were developed using different combination of mAbs. All mAbs showed promising affinities to be used for diagnosis. The sensitivities of ICTs were evaluated using recombinant PfTPx-1 whose results lead us to the preparation of 4 different ICTs. These tests showed positive reaction with *P. falciparum in vitro* culture supernatant indicating the release of PfTPx-1 during schizont rupture. Altogether, these findings suggest that PfTPx-1 is a promising biomarker to diagnose *P. falciparum* infection. However, the diagnostic performance of this antigen should be further validated using clinical samples.

1. Background

Despite being preventable and treatable, malaria still remains as a major public health concern in the world, with 1,238,000 global deaths in 2010 (Murray et al., 2012). The emergence of drug resistance in the malaria parasite, insecticide resistance among the mosquito vectors and the unavailability of an efficient vaccine against malaria are important obstacles for controlling this parasitic disease.

Although parasite/parasite antigen-based diagnosis is increasing, most suspected cases in endemic areas are treated based on presumptive diagnosis (World Health Organization, 2011). Prompt and accurate diagnosis and treatment of the patients with appropriate antimalarial are essential components of malaria control and elimination strategies. Therefore, since early 2010, the WHO has recommended prompt parasitological confirmation by microscopy or rapid diagnostic test (RDT) for all suspected malaria patients before starting the treatment (World Health Organization, 2011). Malaria RDTs were introduced in early 1990s and recently, they have greatly enhanced the quality of malaria diagnosis in endemic areas (Zhao et al., 2012). These RDTs which are lateral flow immunechromatographic tests (ICT), detect parasite antigens by specific monoclonal antibodies (mAbs). Commercial RDTs target one of three antigens, namely histidine rich protein 2 (HRP-2), plasmodial lactate dehydrogenase (pLDH) and aldolase. Despite high sensitivity and specificity for *Plasmodium falciparum* infections, commercial RDTs have known deficiencies such as variable detection thresholds especially in low transmission areas (McMorrow et al., 2011; The malERA Consultative Group on Diagnoses and Diagnostics, 2011). There is a need, therefore, to improve current diagnostic techniques and to develop RDTs targeting additional antigens to address the current deficiencies as well as new challenges in malaria control.

Peroxiredoxin (Prx) is an ubiquitous family of antioxidant enzymes with molecular size of 20–30 kDa that are present in organisms from all kingdoms (Rhee et al., 2005). In different parasites, it is shown that Prxs may be potentially valuable candidates for drugs and vaccine targets (reviewed by Gretes et al., 2012). In addition, Prxs may have diagnostic value for the detection of *Leishmania* spp., *Echinococcus granulosus*, *Fasciola gigantica*, *Taenia* spp. (reviewed by Gretes et al., 2012) and *Schistosoma japonicum* (Angeles et al., 2011, 2012). The cytoplasmic Prxs from *P. falciparum*, *P. vivax* and *P. knowlesi* have been characterized by our group (Hakimi et al., 2012, 2013a; Kawazu et al., 2008). It was shown that *P. falciparum* thioredoxin peroxidase 1 (PfTPx-1) is constitutively and highly expressed through the erythrocytic cycle (Kawazu et al., 2001; Yano et al., 2005), making it a promising candidate as a diagnostic antigen for malaria diagnosis. In this study, we produced several mAbs against PfTPx-1 and evaluated their potential to be used in RDTs.

2. Materials and methods

2.1. Production and selection of mAbs

Recombinant PfTPx-1, *P. vivax* TPx-1 (PvTPx-1) and *P. knowlesi* TPx-1 (PkTPx-1) proteins were expressed as a fusion protein with N-terminal histidine-tag and purified (Hakimi et al., 2012). mAbs were produced as previously described (Hakimi et al., 2013b). Briefly, BALB/c mice were immunized by rPfTPx-1 and hybridomas were developed by fusion of harvested splenocytes to SP2/0 myeloma cells. Single step hypoxanthine-aminopterin-thymidine (HAT) selection using methylcellulose and cloning of hybridoma was performed as previously described, with some modifications (Davis et al., 1982). Hybridoma cloning medium consisted of GIT medium (Nihon Pharmaceutical Co., Tokyo, Japan) containing 5% fetal bovine serum, 5% BriClone (NICB, Dublin, Ireland), HAT and 1.75% methylcellulose. Following 7–10 days incubation, hybridoma clones were picked and grown in wells of 96 well tissue culture microplates and screened using enzyme linked immunosorbent assay (ELISA) and Western blot. The animal experiments in this study were carried out in compliance with the Obihiro University of Agriculture and Veterinary Medicine Guidelines for Animal Experimentation (25–74).

2.2. Purification of mAbs

Isotyping of mAbs was performed using IsoStrip mouse monoclonal antibody isotyping kit (Roche Diagnostics, Indianapolis, IN). IgG and IgM mAbs from hybridoma culture supernatant were purified using protein G (GenScript, NJ, USA) and protein L (GenScript, NJ, USA), respectively, according to manufacturer's instructions. The purity of the mAbs was evaluated by SDS-PAGE under reducing conditions.

2.3. Measurement of binding affinities of mAbs by ELISA

The binding affinities of mAbs were determined by measuring the dissociation constant (K_d) as described before (Friguet et al., 1985). Briefly, constant amounts of mAbs were incubated with various concentrations of rPfTPx-1 until the equilibrium was reached. The mixture of antigen-antibody was then transferred to microtiter plates previously coated with rPfTPx-1 and the remaining unsaturated mAbs were measured by indirect ELISA. K_d was determined using Klotz plot (Friguet et al., 1985).

2.4. Preparation of immunochromatographic tests

Fifty μg/ml of mAb A1 or E4 (Table 1) was added gently to 1 ml of gold nanoparticles (BBI Solutions, Cardiff, UK), then mixed and kept for 10 min at room temperature for the immobilization of antibodies onto the gold nanoparticles' surfaces by physical adsorption. After immobilization, 10 μl of 5% (w/v) polyethylene glycol (PEG) and 100 μl of 10% (w/v) bovine serum albumin (BSA) solution were added to block the non-coated gold nanoparticles' surfaces. After the immobilization and blocking procedures, gold nanoparticle-conjugated mAb was separated by centrifugation. The gold nanoparticle-conjugated mAb was pulse-sonicated for a few seconds and was washed with 1 ml of PBS containing 0.05% (w/v) PEG and 0.5% (w/v) BSA. After mixing, gold nanoparticle-conjugated mAb was collected by the same process as described above. After pulse sonication, the gold nanoparticle-conjugated mAb solution was diluted

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