

14.5 kDa PROTEIN OF *PLASMODIUM FALCIPARUM* IS A SPECIFIC ASEXUAL STAGE ANTIGEN RECOGNIZED BY POOLED OF MOUSE IMMUNE SERA

Heny Arwati, Kusmartisnawati, Yoes Prijatna Dachlan

Department of Parasitology

Airlangga University School of Medicine and Tropical Disease Center Airlangga University

Surabaya, Indonesia

ABSTRACT

The genetic mutation in malaria parasite strain causing diversity characteristic of each strain. Many of the antigens, that P. falciparum express during their life cycle, particularly the asexual blood-stages, are antigenically diverse. The two major causes of antigenic diversity are allelic polymorphisms and antigenic variation. Each stage has different antigens that lead to protective immunity and in many cases; these antigens are not expressed at other stages of the life cycle. The TS.Ag isolated from P. falciparum 2300 strain containing mature asexual stages of parasite. Characterization of this antigen by SDS-PAGE and western blott methods has been performed to find out a malaria vaccine candidate for Indonesia locally. Comparison of the protein content of TS.Ag with R.Ag (ring form stage antigen) and RTS.Ag (ring form, trophozoite and schizont antigen) showed that, 14.5 kDa protein present in these three antigens. Western blott analysis of these antigens showed that, only 14.5 kDa protein of TS.Ag and R.Ag were recognized by mouse polyclonal antibody specific to P. falciparum asexual stage antigen, but not by pooled of malaria falciparum-infected human sera. The 14.5 kDa protein was similar to that of P. falciparum merozoit surface protein-8 (MSP-8) located on the parasitophorous vacuole of ring form and schizont stages of parasite.

Keywords: *Plasmodium falciparum* asexual stage antigen, specific protein

Correspondence: Heny Arwati, Department of Parasitology, Airlangga University School of Medicine and Tropical Disease Center Airlangga University, email : arwatheny@yahoo.com

INTRODUCTION

Malaria is considered a re-emerging disease, due largely to the spread of drug-resistant parasite strains, decay of health-care infrastructure and difficulties in implementing and maintaining vector control programs in many developing countries. Because of malaria's growing global burden, its control is essential. Historically, vaccines have been one of the most cost-effective and easily administered means of controlling infectious disease, yet no malaria licensed vaccine exist for malaria. Accumulating basic and clinical research that effective vaccines for malaria can be developed and could significantly reduce morbidity and mortality, and potentially reduce the spread of infection (James et al, 2007). In fact, heterogeneity of individual response to malaria parasite is affected by intensity of parasite exposure, genetic factor of host/parasite, factor ethnic, geographic and parasite strain (Modiano, 1996). Due to those reasons and due to the complexity of parasite life cycle causing the effective and global vaccine is difficult to produce. Alternatively, is to produce local malaria vaccine which could be applied to Indonesia locally.

However, the genetic mutation in parasite strain causing diversity characteristic of each strain (Sherman, 1998). Many of the antigens, that *P. falciparum* express during their life cycle, particularly the asexual blood-stages, are antigenically diverse. The two major causes of antigenic diversity are allelic polymorphisms and antigenic variation (Anders, 1991). Each stage has different antigens that lead to protective immunity and in many cases, these antigens are not expressed at other stages of the life cycle (Hoffman and Miller, 1996).

Therefore, the characterization of the antigen should be performed herein. The *P. falciparum* asexual stage antigen extracted from Indonesian isolate of *P. falciparum* 2300 strain, has been characterized by SDS-PAGE and western blotting methods to find out the protein content of this antigen. Through this research we are trying to develop a malaria vaccine for being applied to Indonesia locally. This research is an early step of the main research in malaria vaccine development.

MATERIALS & METHODS

Parasite cultivation and synchronization

Parasite used in this study was *P. falciparum* 2300 strain. Parasites were grown in an in vitro culture system according to the method of Treger and Jensen (1976) with some modifications. Parasites were cultured in a 60 mm petridish containing 5% hematocrit of type O human red blood cells (RBCs) in RPMI 1640 medium (Gibco, New York), 0.2% sodium bicarbonate and 10% of type O human plasma. The culture was kept in a candle jar and placed in a 37°C incubator. When the culture reached about 10% parasitemia and majority was ring form stage, culture was then synchronized using 5% sorbitol (Lambros and Vandenberg, 1978). Parasites were then cultured for 20 hrs prior for collecting the mature schizont-infected red blood cells (Schizont-IRBCs).

P. falciparum asexual stage antigen

Schizont-IRBCs rich culture was harvested, hemolysed with 0.15% saponin to collect parasites. After washing with phosphate buffer saline (PBS), freed parasites were then sonicated for 5 minutes in the presence of 1mM phenylmethylsulfonyl fluoride (PMSF), 0.1 mM tosyl-L-lysine chloromethyl ketone (TLCK) and 2mM EDTANA followed by centrifugation at 100.000 g, 4°C for 45 minutes. The supernatant was used as antigen. Protein content of this crude antigen was determined according to the Lawry method. Due to its content of trophozoite and schizont stages of parasite, the antigen is termed TS.Ag. The R.Ag was an antigen prepared from parasite culture, after synchronization mostly ring form-IRBCs was treated as same as TS.Ag. The RTS.Ag was prepared without synchronization. This

antigen contained ring form trophozoite and schizont stages, was then treated as above.

Antibodies

Two kinds of antibody were used in western blott reaction. First, is the pooled of mouse polyclonal antibody obtained from *P. falciparum* asexual stage-immunized mice. Second, a pooled of sera from malaria falciparum-infected patients from malaria endemic area of Nusa Tenggara Barat Province, Indonesia.

SDS-PAGE and Western Blott

SDS-PAGE was done at 30mA for 5 hours, followed by staining with Choomasi blue. Prior western-blotting antigen was reacted with mouse polyclonal antibody against *P. falciparum* asexual stage antigen and also malaria falciparum-infected human sera from malaria endemic areas of Nusa Tenggara Barat Province. The secondary antibody was *Goat anti mouse IgG-horse radish phosphatase-conjugated antibody*. The reaction was followed by substrate o-phenilendiamine (OPD) and stained with AgNO₃. The molecular weight of the bands was confirmed using Protein Ladder Plus (Fermentas, Canada).

RESULTS

Protein content of TS.Ag, R.Ag, and RTS.Ag

Protein fragmentation of the three antigen of *P. falciparum* by SDS-PAGE resulted in the similar protein content of these antigens; these are 130, 73, 56, 55 and 14.5 kDa proteins (Figure 1). Except the TS.Ag contained 96 kDa protein.

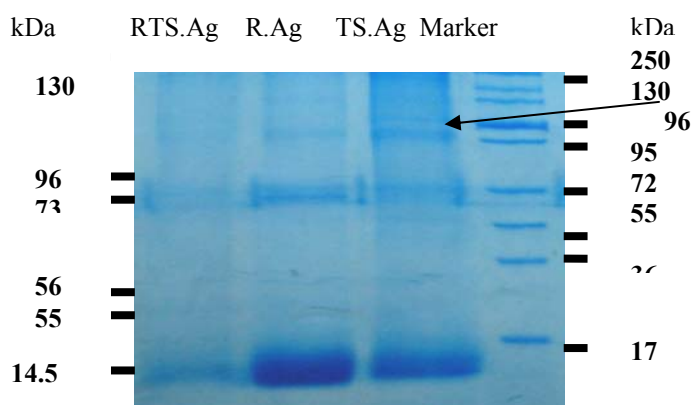


Figure 1. Protein analysis by SDS-PAGE of TS.Ag, R.Ag and RTS.Ag.

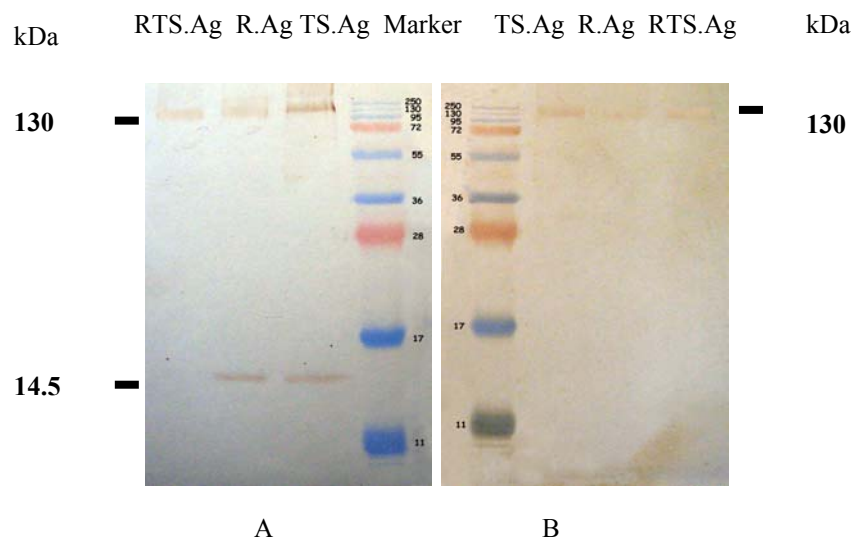


Figure 2A. Specific protein of TS.Ag and R.Ag recognized by mouse polyclonal antibody specific to *P. falciparum* mature asexual stage antigen is 14.5 kDa. Protein 130 kDa is a common epitope of these three antigens. Figure 2B. Neither 14.5 kDa nor 130 kDa protein recognized by malaria *falciparum*-infected human serum from malaria endemic area of NTB Province.

Specific protein recognized by mouse polyclonal antibody specific to *P. falciparum*-asexual stage antigen

The result of western blotting reaction is shown in Figure 2. The 14.5 kDa protein is a specific protein of TS.Ag and R.Ag. This protein was recognized by mouse polyclonal antibody specific to *P. falciparum* asexual stage antigen, but was not recognized by human serum. Interestingly, 96 kDa protein, which was specific protein of TS.Ag was not recognized by this polyclonal antibody, neither by human serum. While, 130 kDa protein, which was present in these three antigens was recognized by both mouse polyclonal antibody and human serum

DISCUSSION

The three antigens contained 130, 73, 56, 55 and 14.5 kDa proteins. Protein 130 kDa was a common epitope of these three antigens, because it was present in the three antigens and recognized by either mouse polyclonal antibody specific to *P. falciparum* mature asexual stage antigen or malaria *falciparum*-infected human serum from endemic area of NTB Province. Only RTS.Ag contained 96 kDa protein, this protein was specific for TS.Ag although was not recognized by mouse polyclonal antibody specific to *P. falciparum* mature asexual stage antigen.

The protein of 14.5 kDa was present in the three antigen. Interestingly, only 14.5 kDa protein of TS.Ag and R.Ag, but not 14.5 kDa protein of RTS.Ag, were recognized only by mouse polyclonal antibody, but not by malaria *falciparum*-infected human serum. During the process of antigen preparation, TS.Ag was prepared from trophozoite and schizont stages of *P. falciparum* 20 hours culture post synchronizarion. On the other hand, R. Ag (mature stage) was only contained ring-form stage (immature stage) post synchronization. While, RTS.Ag was prepared from asynchronized culture, therefore, this antigen contained ring form, trophozoite and schizont stages of parasite. Nevertheless, in fact, the 14.5 kDa protein present in these three antigens. After synchronization process only ring form stage can survive and able to grow synchronizely. This protein is similar to that of *P. falciparum* merozoit surface protein-8 (PfMSP-8). The MSP-8 contains 80 and 17 kDa proteins. Localization of PfMSP-8 by indirect immunofluorescent assay (IFA) throughout the erythrocytic cycle highlights the dynamic nature of the parasitophorous vacuole of ring form stage parasites. In early ring form stage, PfMSP-8 is located in discrete clusters around the circumference of parasites, in an area broadly defined as the parasitophorous vacuole. As parasites mature through the erythrocytic cycle, the level of MSP-8₈₀ decreased. In contrast, MSP-8₁₇ was detected until the schizont stage, especially in the food vacuole of this stage (Drew et al, 2005).

Now, it becomes clear why 14.5 kDa protein present in TS.Ag, R.Ag and RTS.Ag. The mouse polyclonal antibody specific to *P. falciparum* asexual stage antigen did not recognize this protein in RTS.Ag, because during the process of this antigen preparation the IRBCs was not synchronized, therefore, it contained mixture of many protein antigens. The next question is why malaria falciparum-infected human serum did not recognize this protein. The serum of *P. falciparum*-infected patient is specific to *P. falciparum* but did not specific to 14.5 kDa protein. The 130 kDa protein was present in the three antigens and recognized by both mouse polyclonal antibody specific to *P. falciparum* asexual mature stage antigen and malaria falciparum-infected human serum from NTB Province. The 120-250 kDa protein is a Band 3 protein present on the erythrocyte membrane (Murphy et al, 2003). *P. falciparum* protein relates to Band 3 is a protein located on the parasitophorous vacuole of *P. falciparum*. This protein relates to the surface of merozoite when the schizont ruptures. This protein is called acidic basic repeat antigen or ABRA (Kushwaha, 2003). The *P. falciparum* asexual mature stage antigen used in this experiment was also localized on the surface of merozoit stage (Arwati et al, 2007), therefore, it is interested to be further investigated.

ACKNOWLEDGMENT

We thank Malaria Research Group of Tropical Disease Center for providing the malaria falciparum-infected human sera from malaria endemic areas of Nusa Tenggara Barat Province. This research was supported by Research Project of Medical Research Unit Airlangga University School of Medicine, Surabaya, Indonesia, 2006.

REFERENCES

- Anders, R.F. 1991. Antigenic diversity in *Plasmodium falciparum*. Acta Leiden.60: 57-67.
- Arwati, H., Basuki, S., Ideham, B., Hidajati, S., Kusmartisnawati., Safriah, A., Fitri, L.K., Dachlan, Y.P. 2007. Localizatio of *Plasmodium falciparum* asexual stage antigen by mouse immune sera. Folia Medica Indonesiana. 43 (1): 39-43.
- Drew, D.R., Sanders, P.R., and Crabb, B.S. 2005. *Plasmodium falciparum* merozoite protein-8 is a ring-stage membrane protein that lokalized to the parasitophorous vacuole of infected erythrocytes. Infect and Immune. 73(7): 3912-3922.
- Hoffman, S.L. and Miller, L. H. 1996. A Perspective on Malaria Vaccine Development. In Hoffman, S. L. (ed.). Malaria Vaccine Development. A multi-immune Response Approach. ASM Press. Washington DC. page:1-13.
- James, S., and Miller, L. 2007. Malaria vaccine development. <http://www3.niaid.nih.gov/research/topics/malaria/PDF/malvacdev.pdf> .28 September 2007.
- Kushwaha. A., Perween. A., Mukund. S., Majumdar. S., Bhardwaj.D., Chowdhury. NR., Chauhan. V.S. 2002. Amino terminus of *Plasmodium falciparum* acidic basic repeat antigen interacts with erythrocyte membrane through band 3 protein. Mol. Biochem. Parasitol 122(1): 45-54.
- Lambros, C. and Vandenberg, J.P. 1978. Synchronization of *Plasmodium falciparum* erythrocyte stages in culture. J.of Parasitol.65(3): 418-420.
- Modiano, D., Sirima, B.S., Nebie, I., Diallo, D., Esposito, F. and Coluzzi, M. 1996. Different respons to *Plamodium falciparum* malaria in West African sympatric ethnic groups. Proc. Natl. Acad. Sci. USA 93: 13206-13211.
- Trager, W. and Jensen, J.B. 1976. Human malaria parasites in continuous culture. Science. 193: 673-675