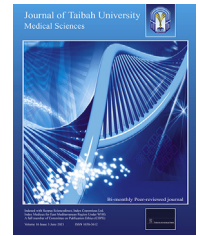Taibah University

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Original Article

Recombinant DBL2β-PfEMP1 of the Indonesian *Plasmodium falciparum* induces immune responses in Wistar rats

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الملخص

أهداف البحث: يعتبر مجال الرابط المشابه دوفي للبروتين١ في غشاء كريات الدم الحمراء للمتصورة المنجلية مسئولا عن الآليات المسببة للمراضة للملاريا الدماغية. والأشخاص الذين يعيشون في المناطق الموبوءة بالملاريا لديهم أجسام مضادة محددة تجاه مجال البروتين ١ في غشاء كريات الدم الحمراء للمتصورة المنجلية وللمناعة للتحكم في شدة الملاريا. يجعل هذا الميل مجال البروتين ١ في غشاء كريات الدم الحمراء للمتصورة المنجلية لقاح محتمل قائم على البروتين. وتهدف هذه الدراسة لاستكشاف الاستجابات المناعية الخلطية والخلوية الناجمة عن مجال الرابط المشابه دوفي ٢ بيتا- للبروتين ١ في غشاء كريات الدم الحمراء للمتصورة المنجلية للبروتين المؤتلف من عزل المتصورة المنجلية الإندونيسية.

طرق البحث: تم التعبير عن البروتين المؤتلف في الإشريكية القولونية كأجزاء قابلة للذوبان وغير قابلة للذوبان، وهذا البروتين تمت تنقيته باستخدام التقارب اللوني قبل الحقن تحت الجلد لفئران ويستر في الأيام ١ و٢١و٤٢. تم حصاد المصل بعد ١٤ يوما من الحقنة الثانية والثالثة لقياس معيار تركيز الغلوبيولين المناعي IgG وCD4+ الخلايا بواسطة تقنية إليزا.

النتائج: وجدنا أن تركيزات الغلوبيولين المناعي IgG وCD4+ الخلايا تزيد بعد الحقنة الثانية والحقن الثالثة. أظهر اختبار مان- ويتني اختلافا كبيرا بين مجموعات التحكم والعلاج لكلا من الغلوبيولين المناعي IgG وCD4+ الخلايا. وأظهر مزيدا من التحليل باستخدام الوصمة الغربية جسما مضادا محددا ضد مجال الرابط المشابه دوفي ٢ بيتا- للبروتين ١ في غشاء كريات الدم الحمراء للمتصورة المنجلية للبروتين المؤتلف.

الاستنتاجات: يمكن لمجال الرابط المشابه دوفي ٢ بيتا- للبروتين ١ في غشاء كريات الدم الحمراء للمتصورة المنجلية للبروتين المؤتلف من عزل المتصورة المنجلية الإندونيسية أن يحفز استجابة مناعية خلطية وخلوية. المزيد من الدراسة على الغلوبيولين المناعي IgG التي تحتمل أن يكون لها تأثير مثبط ودور CD4+ الخلايا وعلاقتهم مع الخلايا المستجيبة الأخرى تعتبر أساسية لتحديد فعالية مجال الرابط المشابه دوفي ٢ بيتا- للبروتين ١ في غشاء كريات الدم الحمراء للمتصورة المنجلية كلقاح الملاريا المحتمل القائم على الببتيد.

الكلمات المفتاحية: CD4+ الخلايا؛ مجال الرابط المشابه دوفي ٢ بيتا؛ الأجسام المضادة IgG؛ إندونيسيا؛ البروتين ١ في غشاء كريات الدم الحمراء للمتصورة المنجلية

Abstract

Objectives: The Duffy binding-like (DBL) domain of the *Plasmodium falciparum* Erythrocyte Membrane Protein 1 (PfEMP1) is reportedly responsible for the pathophysiology of cerebral malaria. People living in endemic malaria areas possess specific antibodies against PfEMP1 and elicit immune responses to control the severity of malaria infection. Therefore, PfEMP1 may be a potential protein-based vaccine candidate. This study aimed to explore the humoral and cellular immune responses induced by the recombinant DBL2β-PfEMP1 obtained from the Indonesian *P. falciparum* isolate.

Methods: The recombinant protein was expressed in *Escherichia coli* BL21(DE3) as soluble and insoluble fractions, and this protein was purified using affinity chromatography before administration as a subcutaneous injection in Wistar rats on days 1, 21, and 42. Sera were harvested 14 days after the second and third injections to determine the titre of IgG and the concentration of

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CD4+ cells using the enzyme linked immunosorbent assay (ELISA).

Results: The IgG titre and the CD4+ cell concentration were found to be increased after the second and third injections. The Mann−Whitney test results showed a significant difference between the control and treatment groups for both the IgG and CD4+ cells ($p = 0.001$ and $p = 0.000$, respectively). Western blotting results indicated the presence of a specific antibody against the recombinant DBL2β-PfEMP1.

Conclusions: The recombinant DBL2β-PfEMP1 of the Indonesian *P. falciparum* isolate could induce humoral and cellular immune responses. Further studies on IgG exerting inhibitory effects and the role of CD4+ cells and their association with other effector cells are essential to determine the efficacy of DBL2β-PfEMP1 and its potential application as a peptide-based malaria vaccine candidate.

Keywords: CD4+ cells; DBL2β; IgG antibody; Indonesia; PfEMP1

Introduction

The fight against malaria, spanning over the last few decades, has been intensified using various programmes of the World Health Organization. The WHO reported a decline in the number of malaria cases throughout the world, owing to the execution of several malaria management programmes in endemic countries.[1] However, with increased usage of insecticide and antiparasitic agents, the problem of resistance has been encountered within highly endemic areas such as those in Asia.[2,3] Furthermore, the use of pesticides and mechanical barriers has created new challenges as these strategies reduce the exposure to the parasite, resulting in a lack of immunity in people in endemic areas.[4,5]

Antibody production remains as a desirable strategy to eliminate malaria infection, particularly in endemic areas. Even though there is no sterile immunity toward malaria infection, studies have shown that people living in endemic regions elicit specific immune responses toward malaria. Clinical immunity allows the affected individuals to control the severity of malaria infection with increasing age.[6] This condition is particularly crucial in cerebral malaria cases which demonstrate high morbidity and mortality in young children. A study conducted in Papua New Guinea showed that a severe cerebral malaria risk is positively associated with age and exposure. Moreover, a high antibody titre against the Duffy binding-like (DBL) domain in adults correlates with a reduced risk of contracted severe cerebral malaria. Presence of a protective antibody reported in adults living in an endemic area has been attributed to the elicitation of an adaptive immune response. It is essential to explore humoral and cellular immune responses against a protein antigen since both aspects of immunity are correlated and affect each other in a reciprocal manner. A potent protein-based vaccine candidate should accommodate both immune responses to provide adequate and lasting protection from infection. A previous study has shown that clinical protection toward malaria infection is dependent on the synergistic association between humoral and cellular immune responses. The cellular immune response component that encounters the antigen triggers cytokine production, further activating other effector cells and inducing antibody production.[7,8] Antibodies generated due to humoral immune response activation provide specific protection toward infection and increase in strength as the exposure is repeated, thereby making immunoglobulin G (IgG) a primary component to be considered during the development of malaria vaccines. Different antigens activate the immune response cascade through various pathways. Protein antigens are mostly T-cell dependent, and an antigen-presenting cell (APC) with a complex protein on its surface known as the major histocompatibility complex (MHC) is necessary to elicit immune responses against protein antigens. APCs project the MHC-bound protein antigen into effector cells, thereby making cellular immune responses such as those involving cluster of differentiation 4+ (CD4+) and CD8+ equally important in the generation of antibodies.[9] Several studies have demonstrated that specific CD4+ cells toward malaria infection are generated in young patients presenting malaria, but an increased CD4+ cell concentration is not always followed by production of clinically functional antibodies to provide protection from other infections.[10]

Plasmodium falciparum Erythrocyte Membrane Protein 1 (PfEMP1) is an attractive target protein in vaccine development against cerebral malaria since this protein plays a critical role in malaria pathogenesis. PfEMP1 is expressed on the surface of infected erythrocytes. It mediates cytoadherence and erythrocyte rosetting, thus obstructing organ vasculatures. The PfEMP1 head structure consists of the Duffy binding-like (DBL) and cysteine-rich interdomain region (CIDR) domains with various arrangements within its highly polymorphic var genes. The preserved structure of these domains is related to their biologic function, as each domain has specific receptors in the human body.[11,12] Binding of intercellular adhesion molecule-1 (ICAM-1) receptor in the brain vasculature to the DBL2β domain of PfEMP1 contributes to the development of cytoadherence. Infected erythrocyte cytoadhesion is one of the key contributors to the pathogenesis of cerebral malaria.[13,14]

The DBL domain, which exhibits an affinity with ICAM-1, such as DBLβPF11_0521 found in children with severe cerebral malaria in Tanzania, is essential in generation of protective antibodies toward cerebral malaria. A previous study showed that children with severe malaria possessed significantly lower levels of antibodies against DBLβPF11_0521, as antibody responses toward this protein were associated with a 37% reduced risk of developing severe clinical malaria.[15] Coded by more than 60 var genes, the PfEMP1 protein demonstrates a high antigenic switching rate. This feature results in elicitation of limited cross-

reactive immune response among different PfEMP1 var types reported in other endemic regions. However, previous reports have indicated that cross-reaction is observed in several cases. A long-term, broad cross-reactive immune response was reported in travelers from endemic areas. DBL2β-PfEMP1 obtained from the *P. falciparum* Indonesian isolate exhibits the highest significant alignment with DBLβPF11_0521 identified in Tanzanian patients.[16] Hence, elucidation of the immune response against DBL2β-PfEMP1 obtained from the Indonesian isolate is vital to understand whether this domain has the potential to be considered as a protein target for vaccine development against cerebral malaria. This study aimed to explore the IgG titres and CD4+cell concentrations generated in Wistar rats in response to recombinant DBL2β-PfEMP1 injections.

Materials and Methods

Production and purification of recombinant DBL2β-PfEMP1

The expression construct of the DBL2β-PfEMP1 Indonesian isolate domain was created using the pET-30a expression vector as per methods described by Hasanah et al.[17] *Escherichia coli* BL21 (DE3) was used as a host to express the recombinant protein. The bacteria were grown at 37 °C until an optical density (OD) of 0.8 at a wavelength of 600 nm (OD600 = 0.8) was achieved; subsequently, bacteria were induced with 0.1 mM IPTG by incubation for 4.5 h at room temperature 20 °C, under shaking conditions at 200 rpm to maximise aeration.[18,19] Pelleted cells of the bacteria obtained via centrifugation were lysed by sonication, and extraction buffer (NaCl 300 mM and Tris HCl 50 mM, pH 7.5) was added. Centrifugation at $12,000 \times g$ for 30 min was performed to separate the soluble fraction before purification. Crude inclusion bodies were then subsequently harvested by solubilisation using denaturation buffer (NaCl 300 mM, imidazole 5 mM, NaH_2PO_4 50 mM, and urea 8 M pH 8.0) and by centrifugation at $12,000 \times g$ for 15 min to obtain the insoluble fraction.[17,20]

Purification was performed using metal chelate affinity chromatography. Resin QIAexpressionist ™ was purchased from Qiagen (Hilden, Germany). His-tag specifically binds the Ni2+ in a column as described by manufacturer.[21] The recombinant protein was eluted using 1.0 M NaCl in 60 mM imidazole (pH 8.0); further, the eluted protein was desalted and concentrated using a concentrator tube (Thermo Fischer Scientific, Waltham, MA, USA) to obtain a fraction with an approximate concentration of 1 µg/µL. The protein concentration of the fraction was measured using the Bradford assay, and the fraction was visualised using SDS-PAGE.

Immunisation of Wistar rats with the recombinant DBL2β-PfEMP1

Fourteen male Wistar rats, aged 3−4 weeks, were randomly divided into three groups. Rats were immunised subcutaneously three times at 3-week intervals. Each immunisation dose contained 150 µg purified recombinant DBL2β-PfEMP1 dissolved in 200 µL phosphate-buffered saline (PBS). Freund's adjuvant (CFA-IFA, Santa Cruz Biotechnology, Dallas, TX, USA) intended to be used in the study was emulsified with the recombinant protein in the first immunisation and was emulsified with an incomplete adjuvant in the following two immunisations, but the control group was injected accordingly with NaCl 0.9% as physiological saline. Serum samples were obtained from all groups two weeks after the second and third immunisation.[22,23]

Analysis of rat sera using western blotting and ELISA

The generated antibodies from rat sera were analysed using ELISA to determine the IgG antibody titre and CD4+ cell concentration. Affymetrix eBioscience Mouse IgG total Ready-SET-Go!® kit (Vienna, Austria) was used according to the manufacturer's instructions. Plates were coated with diluted coating buffer and incubated overnight at 4 °C; subsequently, plates were washed, and wells of the plates were blocked using the blocking buffer after overnight incubation at 4 °C. A total of 100 µL of two-fold serial dilutions of the samples were added to the blocked wells and titres were detected using 50 µL Detection Antibody. Results were visualised using the TMB substrate solution to catalyse HRP enzymatic reaction and values were obtained by analysis at a wavelength of 450 nm.

Confirmation of the generated rat antibodies in response to the recombinant protein was achieved using western blotting as per methods described above. The recombinant protein was subjected to electrophoresis using a 12.5% polyacrylamide gel with SDS and transferred onto a PVDF membrane. The membrane was blocked with 5% skimmed milk in TBS Buffer (Tris−HCl 50 mM pH 8 and NaCl 150 mM), washed three times with TBS Buffer, and subsequently incubated with 1:250 dilution of the serum samples obtained from each group of rats at room temperature for 60 min. After incubation, repeated washing with TBS Buffer was performed before addition of peroxidase-coupled secondary antibody (goat anti-mouse IgG, Invitrogen, Carlsbad, CA, USA) at a dilution of 1:2500 dilution. The membrane was incubated at room temperature for 60 min. The NBT/BCIP substrate was used to reveal the developed blots.

CD4+ concentration was analysed using the Fine Test Mouse CD4 ELISA Kit (Wuhan, China), according to the manufacturer's instructions. Briefly, the plates were washed before addition of 100 µL of the standard, sample, and control. After aspiration, the plates were washed twice. A total of 100 µL of the concentrated biotin-conjugated antibody working solution (diluted 1:100) was added, and the mixture was incubated for 60 min at 37 °C before performing the washing steps thrice. Subsequently, 100 µL HRP-Streptavidin conjugate was diluted 1:100, added to the mixture, and the mixture was incubated for 30 min at 37 °C. After washing five times, 90 µL TMB substrate was added and the mixture was incubated in the dark for 15 min. Visualisation of the target band was performed after addition of the stop solution, and the

plates were read using the Microplate Reader (R-Biopharm, Darmstadt, Germany) at an absorbance of 450 nm.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 26.0 software. Each dataset was analysed for its normality distribution using the Shapiro−Wilk test and its homogeneity of variance was analysed using the Levene's test; Mann−Whitney test and/or the independent T-test based on the normality test with a 95% confidence interval were further used. The difference between the control and treatment groups for each IgG and CD4+ cell concentration and CD4+ cell concentration between soluble and insoluble fractions were analysed using the Mann−Whitney test because the Shapiro−Wilk normality test results showed abnormal data distribution ($p < 0.05$). Furthermore, the independent T-test was performed to compare soluble and insoluble fractions of IgG concentration in treatment groups due to the normal data distribution obtained based on the Shapiro−Wilk test ($p = 0.501$).
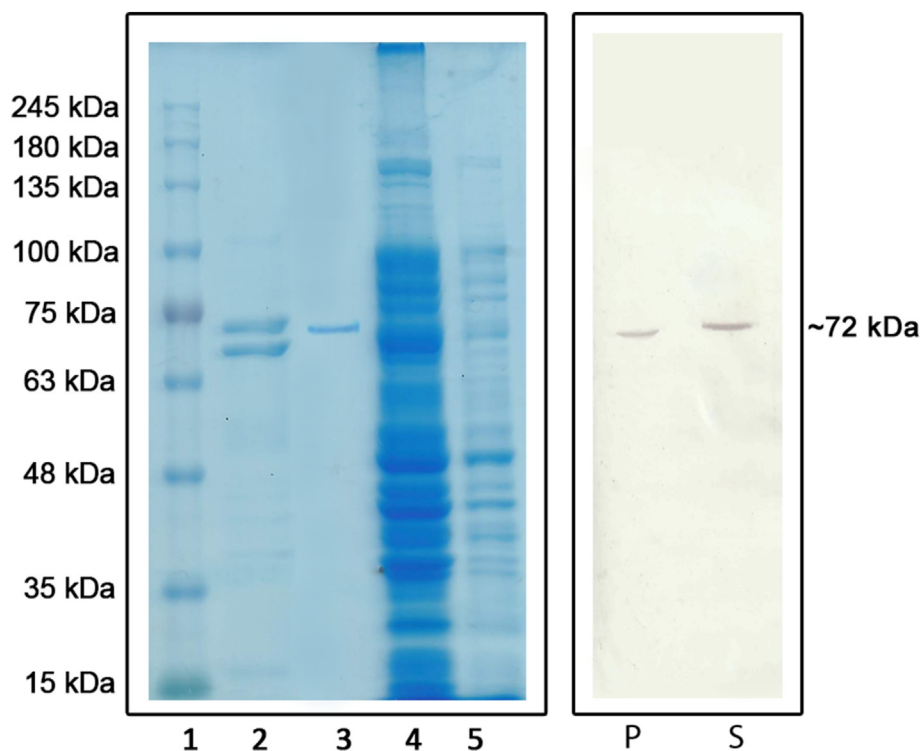
Results

Purified recombinant DBL2β-PfEMP1 was expressed in the soluble and insoluble fractions, after induction with 0.1 mM IPTG for 8 h. Both fractions were obtained as a ∼72-kDa band in SDS-PAGE results. Further confirmation

with western blotting using anti-polyhistidine antibody showed a similar band corresponding to each fraction, as illustrated in Figure 1.

IgG titre in the sera obtained from Wistar rats was measured using ELISA two weeks after administering the purified recombinant protein in the second and the third injections. Figure 2 shows a tabulation of IgG concentration obtained from the analysis of immunised-Wistar rat sera. Results of the analysis using the Shapiro−Wilk normality test for a dataset of control and treatment groups of IgG concentration showed abnormal data distribution ($p = 0.002$). Therefore, the Mann−Whitney U test was performed and results showed that there was a significant difference in IgG concentration between the control and treatment groups ($p = 0.001$). Results of the analysis using Shapiro−Wilk normality test for the determination of the IgG concentration in the soluble and insoluble fractions obtained from the treatment group showed normal distribution ($p = 0.672$ and $p = 0.501$, respectively), and the Levene's test results presented a homogenous variance ($p = 0.791$). Therefore, the independent T-test was performed and results revealed no significant difference between the soluble and insoluble fractions obtained from the treatment groups ($p = 0.214$).

Further analysis using western blotting was performed to confirm the antibody generated as a response toward the recombinant protein injected. Figure 3 illustrates the presence of a single band of ∼72 kDa corresponding to the molecular weight of the recombinant DBL2β-PfEMP1, thereby indicating that the antibody detected was indeed

Figure 1: SDS-PAGE and western blotting results of recombinant DBL2β-PfEMP1. Lane (1): a protein marker; lane (2): purified soluble fraction; lane (3): purified insoluble fraction. Lanes (4) and (5): crude proteins obtained from recombinant *E. coli* and non-transformant *E. coli*. On the right side, western blotting results against anti-polyhistidine antibody, (P): insoluble fraction; (S): soluble fraction.
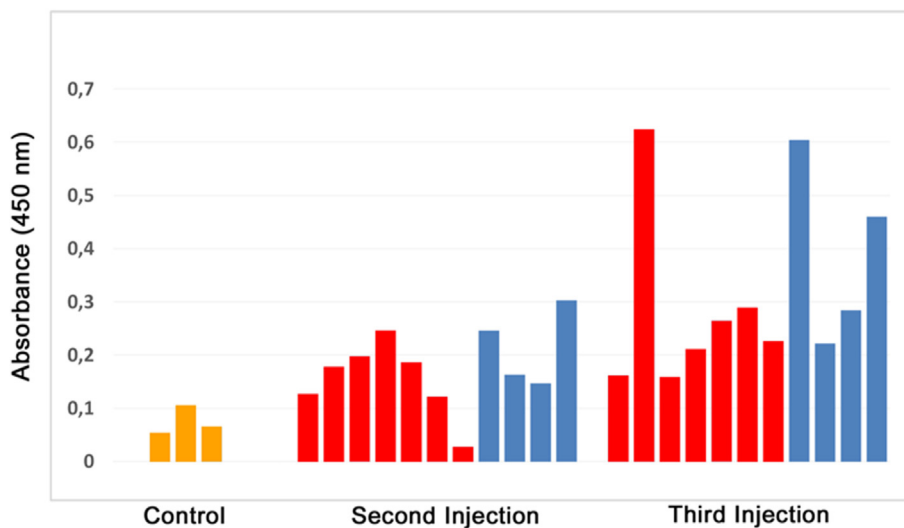
Figure 2: The bar chart shows the IgG antibody concentration presented as optical density for each rat. Orange bars represent the control group, red bars represent IgG in the treatment group that received the soluble protein fraction injection, and blue bars represent IgG in the treatment group that received the insoluble protein fraction injection.

generated as a humoral immune response toward the recombinant DBL2β-PfEMP1.

Cellular immune response was analysed based on CD4+ cell concentration obtained after performing ELISA. Figure 4 illustrates the CD4+ cell concentration measured by using ELISA. There was an increased absorbance observed after ELISA of samples obtained from the treatment groups compared to that of the control group. The Shapiro−Wilk normality test was used and results showed abnormal data distribution (p = 0.000). Hence, the

Mann−Whitney U test was performed and results showed a significant difference in CD4+ cell concentration between the treatment group and the control group (p = 0.000). Moreover, the Shapiro−Wilk test results for a dataset of soluble and insoluble fractions also showed abnormal distribution (p = 0.000 and p = 0003). Furthermore, the Mann−Whitney U test was conducted and no significant difference in CD4+ cell concentration between soluble and insoluble fraction groups was observed in the results obtained (p = 0.577).
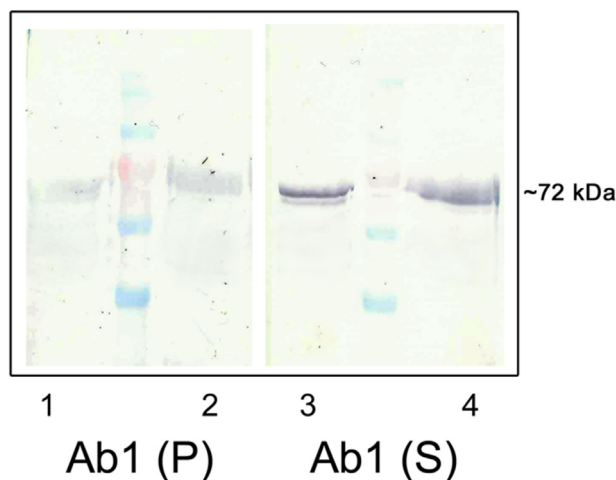
Figure 3: Western blotting results showing a band corresponding to the DBL2β-PfEMP1 recombinant protein. Lane 1: reaction between the pellet protein reacted and sera obtained from rats immunised with the same protein; lane 2: reaction between the supernatant protein and sera obtained from rats immunised with the pellet protein; lane 3: blot reaction between the supernatant protein and sera obtained from rats immunised with the supernatant protein; lane 4: blot reaction between the pellet protein and sera obtained from rats immunised with the supernatant protein.
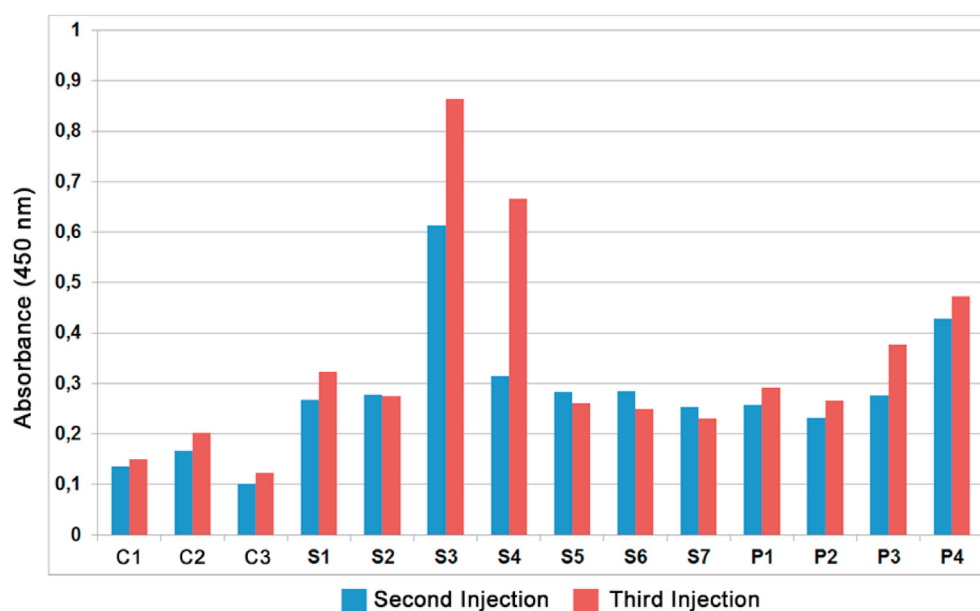
Figure 4: CD4+ cell concentration is presented as absorbance. The blue bars represent the CD4+ cell concentration in serum samples extracted after the second injection, and the red bars represent the CD4+ cell concentration in serum samples extracted after the third injection. C1−C3: control rats, S1−S7: rats injected with the soluble fraction, P1−P4: rats injected with an insoluble fraction.

Discussion

Malaria vaccine development presents with multiple challenges, such as the different stages of parasites, antigen polymorphism, and the immune evasion ability of the parasite.[24] Different approaches to identify target antigens for development of peptide-based vaccines were considered, but currently, few target proteins demonstrate the potential to be used as a vaccine candidate. PfEMP1 is an attractive vaccine target since this protein mediates severe malaria pathophysiology caused by *P. falciparum.* Despite its highly antigenic polymorphisms, conserved areas within PfEMP1 do exist and cross-reaction is observed between isolates, thereby making this protein a potential vaccine candidate.[25]

A vaccine designed using a recombinant protein should exhibit sufficient affinity with MHC molecules to generate an adequate immune response. MHC complexes display the bound antigenic protein on the APC surface to be recognised by lymphocyte T-cells. This study showed that the recombinant DBL2β-PfEMP1 obtained from the Indonesian isolate of *P. falciparum* could induce humoral and cellular immune responses. The generated specific polyclonal IgG antibody was detected in Wistar rats injected with the recombinant protein. Figure 2 illustrates a significant polyclonal IgG concentration difference between treatment and control groups. The specific antibody in sera was also confirmed by western blotting, and the results validated the specific antibody response toward DBL2β-PfEMP1, as indicated in Figure 3.

Reactions between the sera collected from both the treatment groups injected with the soluble and insoluble fractions and the recombinant protein fractions were observed, thereby indicating that this recombinant protein could generate an antibody response despite aggregation in inclusion bodies, possibly due to antigen processing

mechanisms of the host. When a protein antigen enters the host, via natural pathogenic invasion or artificial introduction, it is internalised by an APC and degraded to form short peptides via the action of cathepsins, which are specialised proteolytic enzymes inside the proteasome. Degradation is necessary for the MHC molecule because its limited recognition cleft can only load a specific number of amino acids. Further, a peptide can directly be recognised by B lymphocyte cells without being subjected to the APC processing mechanisms, thereby making it possible for both native and misfolded proteins to stimulate an immune response.[27]

There was also an increase of antibody titre observed after the second injection, and an even higher titre was observed after the third injection, compared with the control group (Figure 2). The IgG antibody titres, which increased gradually from the second injection period to the third injection period, indicated a secondary antibody response generated without the use of complete adjuvants, with higher affinity, and with more specificity to the antigen. The secondary antibody response, known to play an essential role in providing long-term protective humoral immunity against *P. falciparum,* is elicited by the expanded clones of the memory B cells. These cells do not require stimulation or persistent parasite exposure, unlike the plasma cells that generate the primary antibody response after an antigenic challenge.[28]

Similar to the humoral immune response observed, CD4+ cell concentration, as shown illustrated Figure 4, showed a significant difference between the treatment and control group, but no significant difference between the soluble and insoluble fraction groups. These data showed that DBL2β-PfEMP1 could induce a significant cellular immune response. Cellular immunity against blood-stage parasites relies on the activity of CD4+ cells. These cells play a critical role as helper cells for elicitation of CD8+

response and antibody production as well as play a direct effector role in the cellular immune response toward parasites due to their cytotoxic properties. As mentioned in previous studies, specific CD4+ cells play roles as helpers and effectors to control parasitemia in murine models. A certain proportion of CD4+ cells reduce parasitemia and morbidity in malaria patients.[29] CD4+ cells are the key players in elicitation of the cellular immune response to malaria. As previously reported, activated effector CD4+ cells primarily secrete cytokines such as IL-10 and IFN-γ.[30] In malaria patients, cytokine response instability has been observed, possibly due to the antigen's inability to activate CD4+ cells.[31]

Thus far, several malaria vaccine candidates have been identified with a focus on the elicitation of a humoral immune response (i.e., antibody production), although previous studies have reported that antibody production is markedly affected by the cellular immune response. A study conducted in HIV patients infected with malaria showed an association between low levels of CD4+ cells and PfEMP1-specific antibodies, indicating that CD4+ cells could affect antibody induction and maintenance.[32] Only a few vaccine approaches can induce robust production of CD4+ cells; hence, it is critical to identify peptides and develop strategies that can induce production of CD4+ cells.[9,26]

In murine models, CD4+ cells are necessary for the induction of antibody production. Antibodies are the main effectors in a humoral immune response against blood-stage malaria. A high titre of antibodies is vital, especially in the acute stage of infection, to exhibit anti-parasite activity. Furthermore, the quality and functionality of antibodies are also critical.[29,33] Previous studies have shown that antibodies generated in response to PfEMP1 demonstrate a biological function as they can inhibit merozoite invasion, block cytoadherence, and disrupt rosette formation.[11] Adults with PfEMP1 antibodies usually continue to experience *P. falciparum* infection, but exhibit a low level of parasitemia and fewer symptoms. Another study showed that inhibition of rosette formation by the DBL-PfEMP1 antibody could be achieved at a titre 1:500.000.[34]

Previous studies have indicated that naturally acquired antibodies against PfEMP1 can be generated after several infections. The obtained clinical protection depends on parasite strains, since effective immunity against malaria is dependent on various parasite stages and isolates. Typically, naturally acquired antibodies against PfEMP1 exhibit high specificity toward its strain. Thus, a vaccine targeting multiple antigens is necessary to generate a broader clinical protection. Despite the high strain specificity, another study showed that cross-reactive antibodies played the same critical role in mediating protection. *P. falciparum* infection in travelers who returned from endemic areas could generate cross-reactive antibodies with a broad spectrum, and its titre remained for more than 20 weeks post-infection. Certain theories suggest that this can occur due to the presence of shared polymorphic epitopes among PfEMP1 variants and warrant further exploration.[27] A protein antigen binds to MHC molecules through immunodominant epitopes, likely generated from the proteasome antigen-processing phase.

A bioinformatics study of a DBL2β-PfEMP1 Indonesian isolate showed presence of at least two conserved epitopes within its sequence that could be recognised in a broad population in malaria-endemic areas. However, this study did not explore the cross-reactive potential, which has been reported using bioinformatics data in a previously described study. Nevertheless, specific polyclonal IgG antibody induced by the recombinant DBL2β-PfEMP1 in Wistar rats showed that immunodominant epitopes existed within its sequence. The multiple injections of the recombinant DBL2β-PfEMP1 induced both humoral and cellular immune responses. Further studies are necessary to explore its biological function and cross-reactive potential to design an effective malaria peptide-based vaccine.

Conclusion

Our study found that recombinant DBL2β-PfEMP1 of Indonesian *P. falciparum* isolates could induce both humoral and cellular immune responses in Wistar rats after the second and the third injections. The ability to induce a secondary immune response indicated its potential in the generation of antibodies with increased abundance and specificity.

Recommendations

The complexity of the antigen in *P. falciparum* infection is a major challenge in malaria vaccine development. An effective vaccine should induce a secondary immune response, comprising components of both humoral and cellular immunity. Recombinant DBL2β-PfEMP1 showed the ability to induce secondary immune responses, but further studies on the potential IgG inhibiting effect and the role of CD4+ cells and their relation with other effector cells are necessary to determine the suitability of DBL2β-PfEMP1 as a peptide-based malaria vaccine candidate.

Source of funding

Conflict of interests

The authors have no conflict of interests to declare.

Ethical approval

The study received ethical approval on April 30th 2018 with the reference number No.1297/H25.1.11/KE/2018 from The Ethics Committee of Faculty of Medicine, University of Jember, Indonesia. The Indonesian *P. falciparum* isolate was isolated from the blood samples obtained from malaria patients. The study was explained comprehensively to the patients, who then signed a consent form. The animal experiments were performed in accordance with the regulations on animal experimentation.

Authors contributions

SR conducted the study, collected and organised the data, and wrote the initial draft of the manuscript. ES conceived and designed the study, provided research materials and logistic support, analysed the data, and wrote the initial and final drafts of the manuscript. AAIR organised, analysed, and interpreted the data. All authors have critically reviewed and approved the final draft and are responsible for the content of the manuscript.

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