Genetic diversity and immunogenicity of the merozoite surface protein 1 C-terminal 19-kDa fragment of *Plasmodium ovale* imported from Africa into China

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**Abstract**

**Background:** Merozoite surface protein 1 (MSP1) plays an essential role in erythrocyte invasion by malaria parasites. The C-terminal 19-kDa region of MSP1 has long been considered one of the major candidate antigens for a malaria blood-stage vaccine against *Plasmodium falciparum*. However, there is limited information on the C-terminal 19-kDa region of *Plasmodium ovale* MSP1 (PoMSP119). This study aims to analyze the genetic diversity and immunogenicity of PoMSP119.

**Methods:** A total of 37 clinical *Plasmodium ovale* isolates including *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri* imported from Africa into China and collected during the period 2012–2016 were used. Genomic DNA was used to amplify *P. ovale curtisi* (poc) msp119 (pomsp119) and *P. ovale wallikeri* (pow) msp119 (powmsp119) genes by polymerase chain reaction. The genetic diversity of pomsp119 was analyzed using the GeneDoc version 6 programs. Recombinant PoMSP119 (rPoMSP119)-glutathione S-transferase (GST) proteins were expressed in an *Escherichia coli* expression system and analyzed by western blot. Immune responses in BALB/c mice immunized with rPoMSP119-GST were determined using enzyme-linked immunosorbent assay. In addition, antigen-specific T cell responses were assessed by lymphocyte proliferation assays. A total of 49 serum samples from healthy individuals and individuals infected with *P. ovale* were used for the evaluation of natural immune responses by using protein microarrays.

**Results:** Sequences of pomsp119 were found to be thoroughly conserved in all the clinical isolates. rPoMSP119 proteins were efficiently expressed and purified as ~37-kDa proteins. High antibody responses in mice immunized with rPoMSP119-GST were observed. rPoMSP119-GST induced high avidity indexes, with an average of 92.57% and 85.32% for rPocMSP119 and rPowMSP119, respectively. Cross-reactivity between rPocMSP119 and rPowMSP119 was observed. Cellular immune responses to rPocMSP119 (69.51%) and rPowMSP119 (52.17%) induced in rPocMSP119- and rPowMSP119-immunized mice were found in the splenocyte proliferation assays. The sensitivity and specificity of rPoMSP119-GST proteins for the detection of natural immune responses in patients infected with *P. ovale* were 89.96% and 75%, respectively.

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**Background**

Malaria is one of the most severe infectious diseases that threaten human health. According to the World Health Organization, there were 229 million clinical cases of malaria and 490,000 deaths from this disease in 2019 [1]. *Plasmodium ovale* is one of the five species of Plasmodium that regularly infect humans, and accounts for 0.5–10.5% of all malaria cases [2]. It is geographically distributed in sub-Saharan Africa and the Western Pacific Region and is classified into two subspecies: *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri* [2, 3]. *Plasmodium ovale* has a similar morphology and life cycle to *Plasmodium vivax* [2]. The prevalence of *P. ovale* malaria is underestimated because of the low density of these parasites in infected subjects, mild clinical symptoms (e.g. fever), and mixed infections with other species of Plasmodium [2, 4, 5]. However, *P. ovale* infection can evolve into severe malaria with severe anemia and may become fatal, especially in areas where malaria is endemic [6, 7]. Although there have been impressive achievements in research for *P. falciparum* malaria vaccines [8–10], no widely effective vaccine exists for *P. ovale* malaria. China has eliminated malaria within its borders [11], and was very recently certified as officially free of malaria by the World Health Organization. However, as a consequence of economic growth and deepening of the global trade the number of cases of malaria imported into China, including cases of *P. ovale* infection, increased in recent years [12–14].

During the invasion of human erythrocytes by malaria parasites, the merozoite surface proteins (MSPs) (including MSP1) are exposed to the host immune system [15]. Moreover, antibodies targeting MSP1 have been observed in individuals from malaria-endemic areas and have been shown to confer immunity [16–18]. The molecular weight of MSP1 is approximately 200 kDa [19]. The MSP1 protein undergoes two proteolytic cleavage steps during invasion of the erythrocytes. In the first step, MSP1 is cleaved into four polypeptides (with molecular weights of 83 kDa, 30 kDa, 38 kDa, and 42 kDa). Subsequently, the 42-kDa fragment is cleaved into 33-kDa (*MSP1*33) and 19-kDa (*MSP1*19) fragments; the latter remains attached to the merozoite surface and enters the erythrocytes [20–22]. Several studies have identified limited polymorphism in the C-terminal 19-kDa region of both *P. falciparum* MSP1 (PfMSP119) [23–25] and *P. vivax* MSP1 (PvMSP119) [26, 27]. Genetic conservation within vaccine candidate antigens is advantageous for vaccine development as it helps to reduce antigenic escape [27, 28]. Moreover, antibodies against PfMSP119

**Conclusions:** This study revealed highly conserved gene sequences of *pomsp1*19. In addition, naturally acquired humoral immune responses against rPoMSP1 were observed in *P. ovale* infections, and high immunogenicity of rPoMSP119 in mice was also identified. These instructive findings should encourage further testing of PoMSP119 for rational vaccine design.

**Keywords:** *Plasmodium ovale*, Merozoite surface protein 1, Conservation, Immunogenicity
can prevent the invasion of merozoites into erythrocytes [29]. Antibodies to PfMSP119 have been associated with protection from malaria in pregnant women, infants and older children naturally infected with P. falciparum [30–32]. These instructive findings imply that MSP119 is a promising candidate antigen for a blood-stage vaccine.

Genetic polymorphisms of MSP1 in clinical P. ovale isolates from Thailand were analyzed and showed low sequence diversity [33]. However, there is a paucity of information on PoMSP119. In the present study, sequences of pomsp119 from clinical P. ovale curtisi and P. ovale wallikeri isolates from patients with P. ovale malaria imported into China from Africa were investigated. In addition, the immunogenicity of PoMSP119 was assessed in mice, and the levels of immune responses against PoMSP119 assessed in serum samples of patients infected with P. ovale.

**Methods**

**Collection of samples**

Blood samples of febrile patients who had returned from work in malaria-endemic areas of sub-Saharan Africa during the period 2012–2016, and who were confirmed for P. ovale curtisi or P. ovale wallikeri infection by polymerase chain reaction (PCR) tests [13], were obtained from local hospitals in Jiangsu Province, China [34, 35]. Genomic DNA was extracted from the blood samples for PCR amplification of pomsp119 genes. In addition, serum samples of the P. ovale-infected patients (n = 29) who had returned from Africa and those of healthy individuals (n = 20) from China were also obtained from the local hospitals of Jiangsu Province.

**PCR amplification and sequencing of pomsp119**

A total of 37 clinical P. ovale isolates (P. ovale curtisi, n = 20; P. ovale wallikeri, n = 17) were collected for PCR amplification (Additional file 1: Table S1). Sequences of P. ovale curtisi ( poc) msp1 (pomsp1) (KC137343) and P. ovale wallikeri ( pow) msp1 (powmsp1) (KC137341) genes from the GenBank database of the National Centre for Biotechnology Information were used as reference sequences [33, 34]. The 258-bp sequences of pomsp119 were identified via matching with similar sequences, as previously reported [36, 37], and were amplified by nested PCR. The first-round primers were as follows: pomsp119 forward (5’-AGT AAG GAA AAA GAT TTG ACA A-3’), and pomsp119 reverse (5’-AAG TAA GTT AAA TAG GAT GAT-3’). The primers for the nested PCR were as follows: pomsp119 forward (5’-ATG GGA TCT AAA CAT AAA TGT-3’) and pomsp119 reverse (5’-GAA ACC TTC GAA GAA TGG-3’). All the amplification reactions had the same reaction conditions, as follows: 98 °C for 3 min, followed by 35 cycles of 98 °C for 10 s, 45 °C for 1 min, and 72 °C for 1 min, and final extension at 72 °C for 5 min. PCR products were electrophoresed on 1.2% agarose gels for visualization under an ultraviolet transilluminator (ChemiDoc MP; Bio-Rad), then purified and sequenced by Genewiz (Suzhou, China). In addition, PCR-amplified fragments were also cloned into the pUC57 vector and sequenced by Genewiz using the following primers: M13F, 5’-TGT AAA GAC TAA CAC TCA-3’; M13R, 5’-AGG GAA ACA GCT ATG AC-3’.

To evaluate genetic diversity within sequences of different isolates, sequences of pomsp119 and powmsp119 were aligned using GeneDoc version 2.7.0.

**Recombinant expression and purification of proteins**

Genes including pomsp119 and powmsp119 were sub-cloned into the pGEX-6p-1 expression vector which contained a glutathione S-transferase (GST)-tag fusion protein. Recombinant pGEX-6p-1pomsp119 were transferred into Escherichia coli strain BL21 pLysS cells to express PoMSP119 proteins. Colonies of recombinants were cultured in Luria Bertani broth supplemented with ampicillin 50 µg/ml by shaking at 250 r.p.m. at 37 °C until the optical density (OD) at 600 nm reached 0.6–0.8. To induce the expression of recombinant PoMSP119 (rPoMSP119) proteins, isopropyl β-D-1-thiogalactopyranoside (0.1 mM) (TransGen Biotech, Beijing, China) was added and the culture was allowed to grow for another eight hours. Proteins were purified by Tanlen-bio Scientific (Wuxi, China). The rPoMSP119-GST proteins were separated using 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and detected via western blot and Coomassie brilliant blue staining (Beyotime Biotech, China). For western blot analysis, the proteins were electrophoresed on a polyvinylidene difluoride (PVDF) membrane (Immobilon; Millipore Sigma). Nonspecific bindings were blocked by incubation with 5% skimmed milk in Tris-buffered saline supplemented with 0.1% Tween-20 (TBST) at room temperature for 2 h. The membranes were incubated with anti-GST rabbit monoclonal antibody (CWBio Biotech) as the primary antibody at 1:2000 dilution overnight at 4 °C, and then washed three times with 0.1% TBST. Horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin G (IgG) (CWBio Biotech) was used as the secondary antibody for detection (at 1:5000 dilution for 1 h). Finally, the membranes were visualized using the ChemiDoc MP imaging system (Bio-Rad).

**Immunization of mice**

Female BALB/c mice (Cavens; Changzhou, China) aged 6–8 weeks were intraperitoneally injected with 50 µg of rPocMSP119-GST, rPowMSP119-GST, GST, or phosphate-buffered saline (PBS) mixed with Freund's
complete adjuvant (Sigma, San Francisco, CA) as the primary immunization. The same amount of recombinant protein was mixed with incomplete Freund’s adjuvant then injected at days 21 and 42 after the initial injection to boost immunization. Mouse serum samples were collected and stored at −80 °C on days 0, 7, 14, 28, 35, and 49 after the initial injection.

Western blot analysis was performed to detect antibodies directed against rPoMSP119-GST from the sera of immunized mice. Concretely, rPoMSP119-GST and GST proteins were transferred from SDS-PAGE onto PVDF membranes. The membranes were incubated with the sera of mice immunized with rPoMSP119-GST as the primary antibody, with sera of the GST immunized group, or with the sera of those injected with PBS, the negative control group, and then incubated with HRP-conjugated goat anti-mouse IgG (Cowin Biotech) at 1:5000 dilution for detection.

Enzyme-linked immunosorbent assay
Levels of IgG antibodies against rPoMSP119-GST and GST in the sera of immunized mice were evaluated through enzyme-linked immunosorbent assay (ELISA) as previously described [35, 38]. For the assays, 50 ng of rPoMSP119-GST or GST was immobilized on 96-well plates overnight at 4 °C in coating buffer (15 mM sodium carbonate and 35 mM sodium bicarbonate in distilled water) and blocked with 5% (weight/volume) non-fat milk in TBST at room temperature for 2 h. A 100-µl volume of a twofold serial dilution (1:10,000 to 1:5,120,000) of anti-rPoMSP119-GST or anti-GST mouse sera was added to each well and incubated for 1 h at room temperature. The plates were washed three times with PBS containing 0.1% of Tween-20 (PBST) then incubated with HRP-conjugated goat anti-mouse IgG antibodies (Southern Biotech) at 1:5,120,000 dilution for 1 h at room temperature. Finally, the plates were washed again and incubated with 100 µl of 3,3',5,5'-tetramethylenbenzidine (Invitrogen) substrate for 8 min in the dark, and the reaction was stopped with 50 µl of 2 M H2SO4 in each well. The absorbance was read at 450 nm. All samples were tested in duplicate and the mean absorbance was calculated.

The affinity test of the anti-rPoMSP119-GST IgG antibody was performed in accordance with the above-mentioned ELISA test with the exception that the test here was repeated in a 96-well plate coated with the same recombinant proteins. More specifically, sera were incubated for 90 min at room temperature, following by washing of one of the plates with PBST while the other plate was first incubated with 100 µl of TBST containing 6 M urea for 10 min at room temperature before final washing with PBST. Finally, all the plates were incubated with HRP-conjugated goat anti-mouse IgG antibodies at 1:5000 dilution for 1 h at room temperature. The reaction was stopped with 50 µl of 2 M H2SO4 in each well and absorbance was measured at 450 nm. The avidity index (AI) was calculated as follows [39]:

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AI = \frac{(OD_{450}) \text{ of a sample treated with 6Murea/OD}_{450} \text{ of a sample not treated with 6Murea}) \times 100}{100}
\]

Lymphocyte proliferation assays
Assays for lymphocyte proliferation were performed using Cell Counting Kit-8 (CCK-8; Beyotime Biotech), as previously described [35, 40]. Briefly, lymphocytes from mice immunized with rPoMSP119-GST, GST and PBS (5 × 10⁵ cells/well) were treated with 10 µl of rPocMSP119-GST (5 µg/ml), 10 µl of rPowMSP119-GST (5 µg/ml), 10 µl of GST (5 µg/ml) or 10 µl of concanavalin A (ConA; 2 µg/ml) as the positive control in 96-well flat-bottomed microtiter plates then incubated for 72 h at 37 °C with 5% CO₂. A 10-µl volume of CCK-8 was added to each well and the plates were incubated at 37 °C for 2 h. Finally, cell proliferation was measured at 450 nm by a microplate reader.

Screening of serum samples
Serum samples from 29 cases of P. ovale-infected and 20 healthy individuals were screened by well-type amine arrays. The screening was performed as previously described [41, 42]. Briefly, modified glass slides (75 × 25 mm,) were prepared for the protein arrays (CapitalBio, Beijing, China) and warmed to room temperature before use. Teflon tapes with holes were pasted on array glass slides to ensure integrity of the array. One microliter of rPocMSP119-GST, rPowMSP119-GST and GST solution in PBS (100 ng/µl) was spotted into each well of the arrays and incubated for 2 h at 37 °C. Array slides were washed three times with PBST for 10 min and were blocked with 5% of bovine serum albumin in PBST at 37 °C for 2 h. The arrays were washed again and probed with 1 µl of serum samples at 1:200 dilution. Finally, 1 µl of Alexa Fluor 546 goat anti-human IgG (10 ng/µl; Invitrogen) in PBST was added to the arrays for antibody detection. The intensity of the serological responses was measured by a fluorescent microarray scanner (CapitalBio). The positive cut-off value was calculated as the mean fluorescence intensity (MFI) of negative controls plus 2 SD. A Mann–Whitney U-test was performed to compare differences in MFI between groups.
Statistical analysis
GraphPad Prism software (version 5.0; GraphPad Software) was used for the statistical analyses and graphing. An unpaired two-tailed Student’s t-test was used for the comparison of mean values between samples; differences were considered statistically significant when P-values < 0.05.

Results
Genetic description and PCR analysis of pomsp119
The full lengths of PocMSP1 (KC137343) and PowMSP1 (KC137341) were predicted to be 1727 and 1672 amino acids, respectively [34]. Following the proteolytic cleavage of MSP1, schematic diagrams of PocMSP1 and PowMSP1 were divided into seven domains as follows: signal peptide, 83-kDa domain, 30-kDa domain, 38-kDa domain, 33-kDa domain, 19-kDa domain, and glycolphosphatidylinositol (GPI) (Fig. 1a, b). Sequence alignment of amino acids between PocMSP119 and PowMSP119 showed only one amino acid difference for each (amino acids located at positions 1640 and 1585 in PocMSP1 and PowMSP1, respectively) (Fig. 1c). Clinical P. ovale curtisi and P. ovale wallikeri isolates (n = 20 and n = 17, respectively) that were used as sources of genomic
DNA for PCR amplification are listed in Additional file 1: Table S1. PCR products of pomsp19 genes were successfully amplified and showed a single 258-bp band on electropherograms (Fig. 2a). Alignment of pomsp19 sequences from all isolates showed no amino acid mutation (Additional file 2: Fig S1), suggesting that pomsp19 was completely conserved across the isolates.

**Expression, purification, and analysis of rPoMSP119-GST proteins**
The molecular weight of rPoMSP119-GST was estimated as approximately 35 kDa (including the molecular weights of PocMSP119/PowMSP119 and pGEX-6p-1 expression vector containing a GST-tag fusion protein, estimated as 9 and 26 kDa, respectively). Recombinant proteins were efficiently expressed and purified, as shown in Fig. 2b, and presented in SDS-PAGE as a single band of approximately 37 kDa. Western blot analysis confirmed the expression of rPoMSP119-GST proteins (Fig. 2c).

**Mice-derived antibodies against rPoMSP119-GST recognized the recombinant proteins**
To verify whether anti-rPoMSP119-GST antibodies were produced in the sera of immunized mice, we performed an immunoblot for a specific ~37-kDa band of purified rPoMSP119-GST proteins. As expected, sera from mice immunized with rPocMSP119-GST and rPowMSP119-GST detected rPocMSP119-GST and rPowMSP119-GST, respectively (Fig. 3a, b). In addition, sera from mice immunized with GST could also recognize the recombinant proteins (Fig. 3c). No band was found for the recombinant proteins treated with sera from mice immunized with PBS (negative control). These results indicated that rPoMSP119-GST could induce anti-rPoMSP119-GST antibodies in mice.

**Levels of immune responses in mice immunized with rPoMSP119-GST**
To measure levels of immune responses against rPoMSP119-GST or PBS (negative control) in mice, ELISA was performed using rPoMSP119-GST and GST proteins as the coating antigens. The results showed that both rPocMSP119-GST and rPowMSP119-GST were immunogenic (Fig. 4). The average antibody titers were determined by ELISA at 49 days after the first intraperitoneal injection. The proteins rPocMSP119-GST and rPowMSP119-GST induced comparable antibody responses, with end-point titers ranging from 1:10,000 to 1:2,560,000 (Fig. 4a). After three consecutive immunizations, high IgG antibody responses were induced in mice immunized with rPoMSP119-GST (Fig. 4b, c). The titration curves for GST were indicative of low responses compared to those for rPoMSP119-GST proteins. In addition, in comparison to immunization with rPoMSP119-GST proteins, GST immunization failed to induce high IgG antibody responses in mice, and PBS induced no IgG antibody response at all. These results suggested that immunization of mice with rPoMSP119-GST proteins induced higher levels of IgG. 

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![Fig. 3](image_url) **a**–**d** Detection of antibodies in the sera of mice immunized with rPoMSP119-GST. **a**–**c** Western blot analyses for the detection of rPoMSP119-GST proteins by using antibodies from the sera of mice immunized with rPoMSP119-GST and GST proteins. **d** Western blot analysis using antibodies from the sera of mice immunized with PBS. 1 Purified rPocMSP119-GST protein, 2 purified rPowMSP119-GST protein, 3 purified GST protein. For abbreviations, see Figs. 1 and 2.
antibodies (rPocMSP119-GST, mean avidity, 92.57%, \( P = 0.0024 \); rPowMSP119-GST, mean avidity, 85.32%, \( P = 0.014 \)) than immunization of mice with GST (mean avidity, 37.84%) (Fig. 4d). However, there was no statistically significant difference in immune response between the rPocMSP119-GST and rPowMSP119-GST groups of mice (\( P > 0.05 \)).

Cellular immune responses induced by rPoMSP119-GST were assessed through spleen lymphocyte proliferation assays. The proliferation of splenocytes in vitro under rPocMSP119-GST, rPowMSP119-GST, GST, and ConA (positive control) stimulations was determined. Cell proliferation induced by rPocMSP119-GST was 69.51% and that induced by rPowMSP119-GST was 52.17%. Collectively, rPoMSP119-GST proteins induced a stronger proliferation response of spleen cells (rPocMSP119-GST, \( P = 0.0471 \); rPowMSP119-GST, \( P = 0.026 \)) than ConA. GST failed to induce cell proliferation (\( P < 0.0001 \); Fig. 4e).

Cross-reactivity of rPoMSP119-GST proteins with anti-rPoMSP119-GST antibodies

We examined cross-reactivity between rPocMSP119-GST and rPowMSP119-GST using ELISA. rPocMSP119-GST could recognize IgG of sera from mice immunized with rPowMSP119-GST (Fig. 5a, d), with no significant difference observed in cross-reactivity and avidity indices of cross-reaction (\( P > 0.05 \)). However, anti-GST IgG antibodies showed a significantly lower level of cross-reactivity and avidity indices of cross-reaction with rPocMSP119-GST than in reaction of rPocMSP119-GST with anti-rPocMSP119-GST IgG (\( P < 0.001 \); Fig. 5a, d). In addition, the cross-reactivity and affinity of anti-rPoMSP119-GST antibodies and rPowMSP119-GST showed no significance difference in comparison to the reaction of rPowMSP119-GST with anti-rPowMSP119-GST antibodies (Fig. 5b, e). Unexpectedly, rPocMSP119-GST and rPowMSP119-GST showed a significantly higher cross-reactivity and affinity than those of GST with anti-GST
antibodies ($P < 0.05$) (Fig. 5c, f). These results suggested that either rPocMSP119-GST or rPowMSP119-GST had the ability to recognize and bind with high-affinity antibodies induced in mice immunized with rPoMSP119-GST.

**Humoral immune responses to rPoMSP119-GST in P. ovale infections**

Naturally acquired humoral immune responses in patients infected with *P. ovale* were assessed against rPoMSP119-GST. Protein microarrays were used to screen anti-rPoMSP119-GST antibodies in 29 serum samples from *P. ovale*-infected individuals and 20 serum samples from healthy individuals (Additional file 3: Table S2). Patients infected with *P. ovale* showed significantly higher MFI of total IgG against rPocMSP119-GST and rPowMSP119-GST than healthy individuals ($P < 0.0001$) (Fig. 6a, b). Anti-rPocMSP119-GST antibodies showed sensitivity and specificity of 89.96% (MFI value of 26/29 patient samples > cut-off value 7092) and 75% (MFI value of 15/20 healthy samples < cut-off value 7092), respectively. However, no statistically significant difference was observed in the MFI of total IgG against GST protein between *P. ovale*-infected and healthy individuals ($P = 0.5964$; Fig. 6c). These results showed that both rPocMSP119-GST and rPowMSP119-GST are targets of signatures of exposure and immunity.

**Discussion**

Molecules of blood-stage malaria parasites are components of interest for the development of effective malaria vaccines [43]. Several merozoite surface proteins are regarded as promising malaria vaccine candidates because they are targets of host immune selection and play essential roles in erythrocyte invasion [28, 44]. In the first rodent model used to study a malaria vaccine, MSP1 was proven to provide protection against challenge infection [45]. That study showed that after
MSP1 undergoes proteolytic cleavage only MSP119 is carried into the invaded erythrocytes. In a later study, antibodies against MSP119 were shown to prevent merozoites from invading erythrocytes in vitro [20]. Antigenic diversity increases the difficulty of developing an effective malaria vaccine, as it helps parasites to escape from host immune responses [46]. In the present study, sequences of pomsp119 were completely conserved in 20 P. ovale curtisi and 17 P. ovale wallikeri clinical isolates. These results are consistent with those from previous studies which reported genetic conservation of msp119 across malaria parasites [33, 36, 37]. Immune-mediated selection pressure is an important mechanism as it may lead to antigenic diversity [47]. In the present study, we found that the C-terminal region of PoMSP119 is under relatively lower selective pressure than the N-terminal of PoMSP1, which has also been shown to have low genetic diversity [34].

Antibodies play an important role in protection against clinical disease. Earlier studies suggested that antibody responses to PfMSP119 might protect children from high levels of blood-stage parasitemia and clinical malaria [32]. In the present study, cross-reaction between rPocMSP119-GST and rPowMSP119-GST was detected. This cross-reaction indicates that rPocMSP119 and rPowMSP119 share similar antigenic determinants and, therefore, PoMSP119 might possess species-specific efficacy as a vaccine candidate. In addition, as rPomSP119-GST protein contains the GST tag, rPocMSP119-GST and rPowMSP119-GST could be detected by using the sera of mice immunized with GST protein as a non-specific control. Of the five classes of immunoglobulins, IgG is well-known as one that plays a critical role in malaria immunity [48]. A significantly higher level of IgG antibodies was detected in the sera of mice immunized with rPoMSP119-GST proteins than in the sera of the control group of mice immunized with GST protein (Fig. 4), which showed that rPoMSP119-GST induced immune responses in mice. There was no significant difference in cross-reactivity between anti-rPocMSP119-GST IgG and anti-rPowMSP119-GST IgG antibodies, while the affinity index of anti-rPoMSP119-GST IgG was higher than that of the GST group in the cross-reactivity and autoantigen binding test (Fig. 5). These findings imply that antibodies induced in mice immunized with rPoMSP119-GST can bind tightly to antigens and tend to mature. Although anti-GST antibodies were induced in mice immunized with GST protein, affinity maturation was not promoted. High-affinity antibodies are advantageous in a number of biological functions [49]. A better understanding of cellular responses can help in the development of effective blood-stage vaccine candidates for malaria. Lymphocytes play a key role in the immune system and have been shown to be specific for immune responses to infectious microorganisms and other foreign substances [50]. Lymphocyte proliferation assays are widely used to determine T cell immune responsiveness to an antigenic stimulus. The current study showed that rPocMSP119-GST could stimulate the proliferation of T cells (Fig. 4f). This result is particularly instructive as it proves that rPocMSP119-GST can induce cellular immune responses in mice, and more importantly, proves the immunogenicity of rPoMSP119-GST in mice.

![Fig. 6 a–c Analysis of humoral immune responses to rPoMSP119-GST in Plasmodium ovale infections. a IgG responses to rPocMSP119-GST in patients infected with P. ovale and in healthy individuals. b IgG responses to rPowMSP119-GST in patients infected with P. ovale and in healthy individuals. c IgG responses to GST in P. ovale-infected patients and in healthy individuals. * P < 0.05, ** P < 0.01, *** P < 0.001; ns P > 0.05. For abbreviations, see Figs. 1, 2 and 4](image-url)
A number of studies have reported serologic analyses that investigated IgG antibodies against specific antigens of *P. falciparum* and *P. vivax* to assess infection incidence and immunity levels [17, 51, 52]; however, the question of response specificity has not been fully explored. In this study, we also analyzed humoral immune responses in *P. ovale* infections. Most of the *P. ovale*-infected serum samples showed positive antibody responses to rPoMSP119-GST, with a high sensitivity (89.96%) and specificity (75%). The differences in immune responses were not related to the presence of GST protein as no significant difference was observed in the MFI of total IgG against GST protein between healthy individuals (Fig. 6). These data confirmed the infection and *P. ovale* IgG against GST protein between significant difference was observed in the MFI of total IgG against GST protein as no specificity (75%). The differences in immune responses were not related to the presence of GST protein as no significant difference was observed in the MFI of total IgG against GST protein between healthy individuals (Fig. 6). These data confirmed the antigenicity of rPoMSP119-GST, which may indirectly reflect or contribute to protection against *P. ovale* malaria infection as humoral immune responses are partly involved in preventing clinical malaria [53]. Seroepidemiological studies have been particularly informative in areas of low transmission where the sensitivity of surveys for prevalence of malaria parasites is impacted by the low number of parasite-infected individuals [54]. Previous studies have used PfMSP119 and PvMSP119 as serological markers to detect the prevalence of malaria parasites [55, 56]. Due to the limited number of serum samples in the present study we were not able to compare differences in the signal of serological responses with respect to other criteria such as gender and parasite density. However, PoMSP119 as a biomarker of exposure is worthy of further study.

**Conclusions**

This study demonstrated that *pomsp119* sequences were completely conserved in clinical *P. ovale curtisi* and *P. ovale wallikeri* isolates. Furthermore, the immunogenicity of rPoMSP119 in mice was comprehensively analyzed. The informative results presented here contribute to the knowledge base for the development of a PoMSP119-based vaccine. In addition, the naturally acquired antibody responses against rPoMSP119 shown here indicate that further study of PoMSP119 could provide new insights for serological research. The cross-reactivity of PoMSP119 with sera from patients infected with *P. falciparum* or *P. vivax* needs to be explored in future studies, as does the clinical protection against malaria afforded by anti-PoMSP119 antibodies.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s13071-021-05086-6.

**Additional file 1: Table S1.** Information on imported Plasmodium ovale curtisi and Plasmodium ovale wallikeri isolates used in this study.

**Additional file 2: Figure S1.** Alignments of amino acid sequences of Plasmodium ovale curtisi and Plasmodium ovale wallikeri MSP119 in all amplified clinical isolates.

**Additional file 3: Table S2.** Information on serum samples from patients infected with Plasmodium ovale used for the microarrays.

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**Authors’ contributions**

YC and JC conceived and designed the study. YBL, GDZ, JC and QBW collected the samples. QWX, SHL, BY and JCL conducted the laboratory work. QWX and KK wrote the manuscript. KK and YFS reviewed the manuscript. WLZ and MSZ analyzed the data. All authors read and approved the final manuscript.

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**Availability of data and materials**

The data supporting the conclusions of this article are included within the article and its additional files. The new sequences identified in this study have been deposited in GenBank with the accession numbers MZ766552-MZ766553.

**Declarations**

**Ethics approval and consent to participate**

This study was approved by the Ethics Committee of Jiangsu Provincial Key Laboratory on Parasite and Vector Control Technology, Jiangsu Institute of Parasitic Diseases (IRB00004221), Wuxi, China. The animal trial was approved by the Animal Ethics Committee of Jiangnan University [JN no. 20180615t0900930 (100)].

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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**Abbreviations**

ELISA: Enzyme-linked immunosorbent assay; GPI: Glycophosphatidylinositol; GST: Glutathione S-transferase; IgG: Immunoglobulin G; MSP1: Merozoite surface protein 1; PCR: Polymerase chain reaction, SDS-PAGE: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis.
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