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Effect of GPI anchor moiety on the immunogenicity of DNA plasmids encoding the 19-kDa C-terminal portion of *Plasmodium falciparum* MSP-1

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SUMMARY

The glycosylphosphatidylinositol (GPI)-anchored *Plasmodium falciparum* merozoite surface protein 1 (MSP-1) is a widely studied malaria vaccine candidate. The C-terminal 19-kDa portion of MSP-1 (MSP-1₁₉) is of particular interest because this polypeptide moiety remains bound to the parasite even after erythrocyte invasion, while the remainder of MSP-1 is shed during invasion. Studies have shown that antibodies against MSP-1₁₉ inhibit merozoite invasion of erythrocytes efficiently, and that MSP-1₁₉ produces protective immunity in mice and monkeys. To investigate the efficacy of MSP-1₁₉ DNA vaccine and role of GPI anchor moiety in the immunogenicity of MSP-1₁₉, we constructed expression vectors that produce MSP-1₁₉ as either secretory or GPI-anchored polypeptide. Both constructs efficiently expressed MSP-1₁₉ in transfected HEK-293 cells. While the recombinant plasmid lacking GPI anchor signal sequence expressed MSP-1₁₉ mainly as secreted polypeptide, that containing GPI anchor signal sequence produced GPI-anchored MSP-1₁₉ on cell surface. In immunized mice, both constructs produced substantial levels of MSP-1₁₉-specific IgG1, IgG2a, IgG2b, IgG3, IgGA and IgM antibodies. In both cases, the IgG1 level was significantly higher than other isotypes. Interestingly, the plasmid containing GPI anchor signal sequence produced significantly higher levels of IgG2a and IgG2b than the plasmid that lacks GPI signal sequence.

Keywords

Plasmodium falciparum; merozoite surface protein 1; 19-kDa C-terminal polypeptide; glycosylphosphatidylinositol anchor moiety; DNA immunization; antibody responses

INTRODUCTION

Malaria caused by *Plasmodium falciparum* is a major global public health problem and is associated with 300–500 million cases of morbidity and 2–3 millions deaths annually (1). Lack of a protective vaccine and development of drug resistant parasites in most parts of the endemic areas have increased the social and economic burdens of the people (1). Therefore, an effective vaccine is of critical importance in combating malaria.

Merozoite surface protein 1 (MSP-1) is a leading candidate antigen in vaccine design against malaria. *P. falciparum* MSP-1 is a polymorphic GPI-anchored protein with molecular mass varying from 185 to 205 kDa in different strains. MSP-1 is a major protein expressed on the

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surface of merozoites and has been shown to play an important role in the invasion of erythrocytes by merozoites (2). During merozoite maturation, MSP-1 is proteolytically cleaved into four fragments of sizes 83, 30, 38 and 42 kDa, which are held together by noncovalent interactions. During invasion of erythrocytes, the C-terminal 42 kDa fragment is further processed into N-terminal 33 and C-terminal 19 kDa polypeptides (3, 4). MSP-1₁₉ remains membrane bound through the GPI anchor moiety with the invaded merozoites, while the remainder of the MSP-1 complex is shed during erythrocyte invasion. The 19-kDa polypeptide (MSP-1₁₉) contains two conserved epidermal growth factor (EGF)-like domains at its C-terminus.

A substantial portion of the erythrocyte invasion inhibition antibodies in immune sera of people in endemic areas is against MSP-1₁₉ (5). *P. falciparum* MSP1₁₉-specific invasion inhibitory activity has been shown to be associated with resistance to reinfection in Kenyans (6). Antibody-mediated protection against parasite infection challenge was produced in animal models immunized with either 42-kDa MSP-1 or MSP-1₁₉ (7–11). Several studies have shown that MSP-1, especially MSP-1₁₉, is a target of protective antibodies against the blood-stage infection (12,13). Therefore, immunization based on the C-terminal portion of MSP-1 is being pursued as potential vaccine against malaria (14,15).

Enhancing immunogenicity of the antigen through novel strategies to increase protective antibody titer is critically important for MSP-1₁₉ to be an effective malaria vaccine. DNA vaccine is one way to improve the immunogenicity. Immunization using plasmid DNA leads to *in vivo* expression of antigens and subsequent induction of both cell-mediated and humoral immune responses (16). The tissue location of the expressed proteins, i.e., localized intracellularly, on plasma membrane or extracellularly, appears to play a crucial role in the efficacy of DNA vaccines. Therefore, it is important to understand the effect of the factors that dictate tissue locations of the expressed proteins for exploiting MSP-1₁₉ as a malaria vaccine antigen through DNA vaccine approach. The presence of a C-terminal GPI anchor sequence and/or an N-terminal peptide sequence in the DNA vaccine antigen leads to the expression of cell surface bound and secretory proteins, respectively.

Previous studies have shown that the *P. falciparum* peptide signal sequence and GPI anchor signal sequences are either nonfunctional or poorly functional in mammalian cells (17,18). Recently, by studying *P. yoelii* PyMSP-4/5, Wang *et al.* have reached a similar conclusion (19). When parasite's signaling sequences were replaced by corresponding mammalian signaling sequences, parasite proteins were efficiently expressed in mammalian cells as GPI-anchored, transmembrane-bound or secretory proteins (19,20). Immunization of mice with the recombinant plasmid constructs has induced high levels of PyMSP-4/5 specific antibodies, but with different isotype distribution (19). In this study, we investigated the expression pattern of two *P. falciparum* MSP-1₁₉ DNA constructs, one containing a mammalian secretory peptide signal sequence at the N-terminus and the human decay-accelerating factor (DAF) GPI signal sequence at the C-terminus, and the other having only a mammalian secretory peptide signal sequence. We have also investigated the immunogenicity of these two DNA plasmid constructs in mice.

MATERIALS AND METHODS

Reagents

Human blood and plasma for *P. falciparum* culturing were from Hershey Medical Center, Hershey. [6-³H]Glucosamine (23 Ci/mmol) was from Amersham Pharmacia Biotech. Alexa Fluor 568-conjugated goat anti-mouse IgG was from Molecular Probes. The nucleotide primers were from Invitrogen. Monoclonal antibody mAb 5.2 against an epitope in the C-terminal

portion of MSP-1 was generously provided by MR4, ATCC. The recombinant MSP-1₁₉, expressed in yeast (21), was provided by Dr. David Kaslow.

Construction of MSP-1₁₉ recombinant plasmids

Culturing of *P. falciparum*, and isolation of parasites and parasite total RNA were performed as reported previously (22). The parasite RNA was reverse transcribed, using a oligo (dT)₁₂₋₁₈ primer and Superscript II reverse transcriptase (Invitrogen), as described (23). cDNA was prepared from total RNA isolated from human peripheral blood mononuclear cells.

The *MSP-1* gene fragment encoding the 142-amino acid residue polypeptide (designated as MSP-1₁₉), corresponding to F¹⁵⁵⁵ through I¹⁶⁹⁶ (three residues upstream of the GPI cleavage site), in the C-terminal portion of the mature *P. falciparum* MSP-1 (NCBI accession number XP_001352170), was amplified from the parasite cDNA using primers 5'-GAAGATCTTTTAAAAACCCCTTACGACTTC 3' (forward; restriction site *Bgl* II is underlined) and 5'-TCCCCCGGGGAATACCATCGAAAAGTGG- 3' (reverse; restriction site *Xma* I is underlined). The MSP-1₁₉ cDNA was cloned into *Bgl* II and *Xma* I sites of pDisplay-ΔTM-Neo plasmid (prepared by deleting *Sal* I to *Xho* I fragment in pDisplayTM plasmid (Invitrogen) to obtain the construct pDisplay-ΔTM-Neo-MSP-1₁₉-Sec (Plasmid P1; see Fig. 1). The gene sequence corresponding to the C-terminal 37 amino acid peptide fragment of human DAF containing GPI signal sequence was amplified from cDNA prepared using total RNA isolated from human peripheral blood mononuclear cells. The primers used were: forward, 5'-TCCCCCGGGGCCAAATAAAGGAAGTGGAAAC 3' (restriction site *Xma* I is underlined), and reverse, 5'-AAAACTGCAGCTAAGTCAGCAAGCCCATGGT 3' (restriction site *Pst* I is underlined). The cDNA fragment was fused downstream of MSP-1₁₉ cDNA sequence of pDisplay-ΔTM-Neo-MSP-1₁₉-Sec (Plasmid P1; see Fig. 1) as outlined above to obtain the recombinant plasmid pDisplay-ΔTM-Neo-MSP-1₁₉-Gpi (Plasmid P2). In both cases, positive clones containing full-length inserts were screened by restriction analysis. The DNA sequences in the constructs were confirmed by automated DNA sequencing.

Transfection of plasmid DNA to HEK-293 cell

Plasmid DNA was prepared using endotoxin-free plasmid Maxi Kit (Qiagen) according to the manufacturer's instructions. HEK-293 cells were cultured in Dulbecco's modified Eagle's medium, supplemented with 10% heat-inactivated fetal bovine serum and penicillin/streptomycin. The cells (1×10^6) were plated in 6-well plates, cultured overnight, and transfected with 2 μg of DNA using FuGENE6 (Roche) transfecting agent according to the manufacturer's instructions. After 20 h, the transfected cells were washed with ice-cold phosphate buffered saline (PBS), pH 7.4, and then lysed with ice-cold immunoprecipitation assay buffer (50 mM Tris-HCl, 150 mM NaCl, pH 7.2, containing 1% sodium deoxycholate, 0.1% SDS, and 1% Triton X-100). The lysates were stored at -80°C until used for Western blotting or SDS-PAGE/fluorography.

Metabolic labeling of cells

HEK-293 cells in 6-well plates were transfected with the plasmids, 12 h later metabolically labeled with [6-³H] glucosamine (50 μCi/mL; Amersham Biosciences) in RPMI 1640 medium containing 5 mM glucose for 20 h. The cells were harvested, lysed, and analyzed by SDS-PAGE/fluorography.

Western blot analysis

HEK-293 cells in 6-well plates were transfected with the recombinant plasmids. After 20 h harvested and suspended in PBS. Cells were lysed in reducing SDS-PAGE sample buffer and

heated at 95°C for 5 min. The cell lysates were electrophoresed on 10% SDS-polyacrylamide gels and blotted onto nitrocellulose membranes. The membranes were blocked with 5% nonfat dry milk in PBS and incubated with 1 µg/mL of monoclonal antibody mAb 5.2 followed by horseradish peroxidase-conjugated goat anti-mouse IgG (Cell Signaling Technology), treated with chemiluminescent substrate LumiGLO (KPL), and exposed to X-ray films. The levels of MSP-1₁₉ on membranes were estimated by measuring the protein band intensity using FUJIFILM Image Station model LAS-3000 and normalizing the band density with respect to that of the 48 kDa band in the respective lane.

Immunofluorescence

HEK-293 cells expressing the recombinant MSP-1₁₉ were cultured as monolayer on glass cover slips placed in 6-well plates, fixed with 2% paraformaldehyde at room temperature for 10 min, permeabilized with 10 mM Tris-HCl buffer, 120 mM KCl, 20 mM NaCl and 1 mM EDTA, pH 8.0, containing 0.1% Triton X-100 at room temperature for 15 min. The cover slips were washed twice with PBS, pH 7.4, blocked with 3% bovine serum albumin, and then incubated for 1 h with a monoclonal antibody (mAb 5.2; 20 µg/ml in blocking buffer). The cover slips were washed three times with PBS, pH 7.4, and then incubated with a mixture of Hoechst 33258 and 1:400 diluted Alexa Fluor 568-conjugated goat anti-mouse IgG. The cover slips were washed again with PBS and mounted on glass slides and examined by fluorescent and confocal microscopy.

Immunization of mice with MSP-1₁₉ recombinant plasmids

A group of five BALB/c mice were immunized intramuscularly with the recombinant plasmid pDisplay-ΔTM-Neo-MSP-1₁₉-Sec, P1 (0.1 mg each per mouse) in TE buffer, a group of another five mice was similarly immunized with plasmid pDisplay-ΔTM-Neo-MSP-1₁₉-Gpi (P2). Five mice were also immunized with the control pDisplay-ΔTM-Neo plasmid (Pv). After 3 and 6 weeks, mice were injected with 0.1 mg of the respective plasmids each time. At the end of ninth week, mice were sacrificed, blood collected, sera prepared, and stored at -80°C.

Assessment of antibody responses in immunized mice by ELISA

Ninety-six well microtiter plates were coated with 50 µL of 2 µg/mL solution of the recombinant MSP-1₁₉ (21) in 50 mM sodium bicarbonate buffer. The plates were incubated at 4°C overnight, washed three times with 10 mM Tris, 150 mM NaCl, pH 7.5 containing 0.05% Tween 20 (TBST), and then blocked with 100 µL of 5% fat-free milk in TBST at room temperature for 2 h. Sera (1:2 serially diluted starting from 1:1500) were added and the plates were incubated at room temperature for 1 h. After washing three times with TBST, the plates were incubated with 1:2000 diluted horseradish peroxidase-conjugated goat anti-mouse IgG at room temperature for 1 h. Finally, the plates were washed and developed with a peroxidase substrate solution SureBlue™ TMB peroxidase substrate (KPL), and the reaction stopped with 2 N H₂SO₄. The absorbance of the developed color was measured at 450 nm. Preimmune serum from each animal was similarly analyzed as control. The ELISA determinations were done in duplicates and the values were plotted as means ± standard deviation. Antibody titers were defined as the reciprocal values of highest dilutions that gave OD values 2 times higher than OD values of background.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 4.0. Significance between values from two immune sera was determined by student 't' test, and p values <0.05 considered significant.

Immunoglobulin isotyping

Immune sera (1:3000 diluted) were analyzed for the antibody isotypes using the mouse immunoglobulin isotyping ELISA kit (BD Pharmingen) according to the manufacturer's instructions. Values are plotted as means $\pm$ standard deviation. Statistical analysis was performed as outlined above.

RESULTS

P. falciparum MSP-1₁₉ recombinant plasmids

To determine the effect of GPI anchor moiety on the immunogenicity of *P. falciparum* MSP-1₁₉, we constructed two recombinant plasmids, based on *MSP1* gene sequence of 3D7 parasite strain, using the strategy outlined in Fig. 1. The expression plasmid (pDisplay™) used was a human CMV promoter-based vector containing murine Igk chain leader sequence (peptide signal sequence) to target the expressed protein to the secretory pathway and HA epitope to allow for detection of the protein. The myc epitope and PDGFR transmembrane domains present in the pDisplay vector were removed by deleting *Sal* I to *Xho* I fragment to obtain a modified vector termed pDisplay-ΔTM-Neo plasmid. The first recombinant plasmid was constructed by inserting the *MSP-1* gene fragment encoding the 142-amino acid C-terminal portion (three amino acid residues upstream of the GPI signal sequence) of MSP-1 into the pDisplay-ΔTM-Neo vector. The *MSP-1*₁₉ fragment was ligated downstream of the peptide signal and HA tag DNA sequence in the pDisplay-ΔTM-Neo vector. This recombinant plasmid, pDisplay-ΔTM-Neo-MSP-1₁₉-Sec, was designated as P1 (Fig. 1). For the construction of the second recombinant plasmid, the C-terminal 37 amino acids containing the GPI signal sequence of human DAF gene fragment was fused at the downstream of *MSP-1*₁₉ into the pDisplay-ΔTM-Neo-Sec vector, to obtain the recombinant plasmid, pDisplay-ΔTM-Neo-MSP-1₁₉-Gpi, which was designated as P2 (Fig. 1). Both P1 and P2 recombinant plasmids contained an MSP-1₁₉ peptide expression cassette fused to the murine Ig κ-chain leader sequence and HA tag at the N-terminus. P2 but not P1 contained a functional mammalian GPI anchor signal sequence. Thus, plasmid P1 was designed to express MSP-1₁₉ as the secretory polypeptide, while P2 to express the polypeptide as the GPI-anchored molecule.

Characterization of MSP-1₁₉ polypeptides expressed in HEK-293 cells transfected with recombinant plasmids

The recombinant proteins expressed by the transfected cells were analyzed by Western blotting using a monoclonal antibody (mAb 5.2), which is specific to an epitope in the C-terminal 19 kDa fragment of *P. falciparum* MSP-1 (24). Consistent with the presence of GPI moiety, the electrophoretic mobility of MSP-1₁₉-Gpi was noticeably slower than that of MSP-1₁₉-Sec (Fig. 2A). The estimated molecular mass of MSP-1₁₉-Sec and MSP-1₁₉-Gpi polypeptides, based on their electrophoretic mobility on gels, corresponded to 16.5 and 19 kDa, respectively. Quantification of protein bands on membranes, by densitometry scanning and normalizing to the 48 kDa band on the respective lane, suggested that MSP-1₁₉-Sec and MSP-1₁₉-Gpi expressed at comparable levels in transfected HEK-293 cells.

To confirm the presence of GPI moiety in MSP-1₁₉-Gpi, the transfected HEK-293 cells were metabolically labeled with [³H]glucosamine and cell lysates were analyzed by SDS-PAGE/fluorography. The cells transfected with P2 expressed a radiolabeled band corresponding to the molecular size of MSP-1₁₉-Gpi, whereas in cells transfected with P1 MSP-1₁₉-Sec was not radiolabeled (Fig. 2B). Thus, these data demonstrated that MSP-1₁₉-Gpi is a GPI-anchored polypeptide. MSP-1₁₉ contains one potential glycosylation site at amino acid residue N¹⁶⁰⁷ of the full length MSP-1. Since MSP-1₁₉-Sec was not radiolabeled with [³H]glucosamine, this N-glycosylation site was not modified with carbohydrate moieties.

To ascertain further whether the mammalian N-terminal peptide and C-terminal GPI signal sequences in the recombinant plasmids were functional, we examined the expression patterns of MSP-1₁₉-Sec and MSP-1₁₉-Gpi polypeptides encoded by plasmids P1 and P2 in transfected HEK-293 cells. Immunofluorescence analysis using showed that both plasmids efficiently expressed the recombinant MSP-1₁₉ polypeptides (Fig. 3). Confocal images revealed that the polypeptide MSP-1₁₉-Sec expressed by P1 was mostly secreted with very little remain in the cytoplasm. Interestingly, almost all MSP-1₁₉-Sec polypeptide was retained in the extracellular matrix since the polypeptide was not detectable in the culture supernatant when analyzed by SDS-PAGE (data not shown). MSP-1₁₉-Gpi polypeptide expressed by P2 localized exclusively to the cell surface. Thus, the above data together established that both the peptide and GPI signal sequences in P1 and P2 plasmids were functional and that the polypeptides expressed by both recombinant plasmids were localized to the expected cellular sites.

Antibody responses in mice immunized with the recombinant plasmids

Sera from immunized mice were analyzed for antibodies to MSP-1₁₉. Both recombinant plasmids produced significant levels of MSP-1₁₉-specific antibody responses (Fig. 4). Of the two recombinant plasmids, P2 that expressed GPI-anchored polypeptide, MSP-1-Gpi, gave significantly higher antibody titer than P1 that expressed secreted polypeptide, MSP-1₁₉-Sec. The mean antibody titers of sera of mice immunized with P1 and P2 were, respectively, 24,000 and 48,000.

Antibody isotypes

Ig isotype analysis of sera from mice immunized with the MSP-1₁₉ recombinant plasmids indicated that both P1 and P2 plasmids produced substantial levels of IgG1, IgG2a, IgG2b, IgG3, IgM, and IgA (Fig. 5). Both plasmids produced significantly higher levels of IgG1 as compared to other Ig isotypes. The MSP-1₁₉ plasmid P2 with fused GPI anchor signal sequence produced significantly higher levels of IgG2a and IgG2b as compared to the plasmid P1 that lacked GPI anchor signal sequence (Fig 5). IgG3, IgA, and IgM were also produced somewhat at higher levels by P2 plasmid than by P1.

DISCUSSION

Immunization with recombinant plasmids that encode *P. falciparum* antigens is one of the strategies for malaria vaccination. Of various factors that determine the efficacy of DNA vaccines, the level of expression, post-translational modifications, and cellular locations of the targeted antigens play important roles. These factors are in turn determined by signal sequences such as the N-terminal peptide signal and/or C-terminal GPI/transmembrane signal sequences. Previous studies have shown that the N-terminal peptide signal and C-terminal GPI signal sequences of *P. falciparum* are either not recognized or poorly recognized by mammalian cells (17,18). When parasite genes containing the endogenous signaling sequences were expressed in mammalian cells, the proteins retained in cytoplasm and degraded (17,18). Studies have also shown that when parasite signaling sequences are replaced by corresponding mammalian signal sequences, the proteins were efficiently targeted to the expected cellular locations and undergo cell-specific posttranslational modifications (19,20). Therefore, a prior knowledge on the expression pattern and posttranslational modifications of the targeted proteins and efficiency of the induced immune responses is important for rational design of malaria DNA vaccines.

In the present study, we investigated the expression of 19-kDa C-terminal portion of *P. falciparum* MSP-1, a potential malaria vaccine candidate, by mammalian cells transfected with recombinant plasmids containing corresponding parasite gene sequence fused to murine peptide signal sequence and/or human GPI signal sequence. The results show that cells transfected with the DNA plasmid containing both N-terminal peptide and C-terminal GPI

signal sequences (P2) is expressed exclusively on the cell surface. In contrast, cells transfected with the DNA plasmid containing the peptide signal sequence but the lacking GPI sequence (P1), the polypeptide was secreted. Thus, in both cases, MSP-1₁₉ was expressed at the expected cellular locations.

MSP-1₁₉ contains one potential *N*-glycosylation site at Asn¹⁶⁰⁷ of full length MSP-1 (Asn⁵⁴ in the MSP-1₁₉ polypeptide) and was predicted to undergo *N*-glycosylation when expressed in mammalian cells. However, this site was not glycosylated as indicated by the absence of radiolabeling in the polypeptide expressed by cells transfected with plasmid P1 when metabolically labeled with [³H]glucosamine. This is despite the fact that a major portion of the polypeptide routed through the secretory pathway and the polypeptide had an opportunity to undergo *N*-glycosylation. The absence of *N*-glycosylation was not due to failure of cells to metabolically label carbohydrates since the polypeptide expressed by P2 plasmid incorporated [³H]glucosamine into the GPI anchor moiety as indicated by its expression on the cell surface. This is interesting because native parasite proteins are not modified with *N*-glycans since the parasite lacks *N*-glycosylation machinery. Thus, both secreted and GPI-anchored forms of MSP-1₁₉ were not *N*-glycosylated, which otherwise expected to dramatically alter the protein structures and their immune responses.

Analysis of the immunogenicity of the recombinant plasmid constructs (P1 and P2) showed that both were immunogenic in mice and produced substantially high levels of anti-MSP-1₁₉ antibodies. Compared to P1 plasmid expressing MSP-1₁₉ lacking GPI moiety (MSP-1₁₉-Sec), the P2 plasmid expressing GPI-anchored MSP-1₁₉ (MSP-1₁₉-Gpi) induced significantly higher levels of antibodies. These observations agree with the results of previous studies. Martinez *et al.* have shown that recombinant *P. berghei* sexual-stage protein, Pbs21, expressed in a baculovirus system as a GPI anchored protein, was markedly more immunogenic than the recombinant protein lacking GPI-moiety (25). Wang *et al.* demonstrated that plasmids encoding *P. yoelii* MSP4/5 as GPI-anchored protein induced higher level of antibodies compared to the plasmid expressing MSP4/5 as transmembrane protein (19). Therefore, the presence of GPI moieties significantly enhances the immunogenicity of parasite proteins.

Assessment of antibody isotypes in immunized mice showed that both recombinant plasmid constructs produced significant levels of various Ig isotypes, IgG1, IgG2a, IgG2b, IgG3, IgGA, and IgM, but with distinctive isotype profiles (see Fig. 5). IgG1 was somewhat lower in mice immunized with P2 as compared to P1, although the difference was not significant. Both recombinant plasmid constructs induced significant levels of IgG1 (induced by Th2 type immune response), and IgG2a and IgG2b (induced by Th1 type immune response). Induction of both Th1 and Th2 type immune responses by the DNA constructs is interesting considering that a balanced Th1 and Th2 responses is thought to be crucial for protective immunity against blood stage malaria (26). Th1 cells produce interferon gamma and tumor necrosis factor, responsible for cell-mediated protective immunity.

As shown in Fig. 5, IgG2a and IgG2b responses were significantly higher in mice immunized with MSP-1₁₉ recombinant plasmid containing GPI anchor signal sequence as compared to that lacking GPI anchor signal sequence. These results agree with those reported by Wang *et al.* that recombinant plasmids containing *P. yoelii* MSP4/5 fused to human DAF GPI anchor signal sequence induced significantly higher IgG2a and IgG2b antibody response as compared to the MSP4/5 construct lacking GPI anchor signal sequence (19). Together, these observations suggest that the plasmids expressing GPI-anchored proteins induce relatively stronger Th1-type immune responses than plasmids encoding proteins without a GPI anchor moiety. Previously, it has been shown that IgG2a response was associated with protection against *P. yoelii* infection (27). Thus, it appears that inclusion of a functional GPI anchor to the MSP-1₁₉ DNA construct induces better protective immune responses.

In summary, in this study, we assessed for the first time the effect of a GPI anchor signal sequence on the immunogenicity of DNA vaccine constructs encoding *P. falciparum* MSP-1 19 kDa C-terminal moiety as GPI-anchored polypeptide. The results demonstrate that the inclusion of a mammalian GPI signal sequence to MSP-1₁₉ DNA vaccine leads to the efficient expression of protein on cell surface and fairly enhanced induction of Th1-type antibody immune responses.

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Figure 1.

Plasmid DNA construction strategy. The 142 amino acid C-terminal portion of *MSP-1* gene was PCR amplified with 5' *Bgl* II and 3' *Xma* I restriction sites and inserted into pDisplay-ΔTM-Neo vector, to generate pDisplay-ΔTM-Neo-MSP1₁₉-Sec (designated as P1). Similarly, The 37 amino acid GPI anchor sequence of human *DAF* gene was PCR amplified with 5' *Xma* I and 3' *Pst* I restriction site and inserted downstream of MSP-1₁₉ in pDisplay-ΔTM-Neo-MSP1₁₉-Sec to generate pDisplay-ΔTM-Neo-MSP1₁₉-Gpi (designated as P2).

Figure 2.

Analyses of MSP-1₁₉ recombinant protein expression in mammalian cells. HEK-293 cells were transfected with the recombinant plasmids P1 and P2, and cultured. (A) Lysates from cells transfected with P1 (lane 1) and P2 (lane 2) were electrophoresed on 10% SDS-polyacrylamide gel, proteins bands transferred onto nitrocellulose membranes, and MSP-1₁₉ polypeptides probed with monoclonal antibody mAb 5.2, which is specific to the 19-kDa C-terminal portion of *P. falciparum* MSP-1. Upper and lower arrows in the right margin indicate, respectively, the positions of MSP-1₁₉-Sec (expressed by P1) and MSP-1₁₉-Gpi (expressed by P2). (B) Cells were metabolically labeled with [³H]glucosamine and lysates were analyzed by SDS-PAGE/fluorography analysis. Lane 1 and lane 2, respectively, indicate cells transfected with P1 and P2. Arrow in the right margin indicates the position of [³H]glucosamine-labeled MSP-1₁₉-Gpi. [³H]glucosamine was not incorporated into MSP-1₁₉-Sec in lane 1, indicating the absence of *N*-glycosylation in the polypeptides.

Figure 3.

Determination of cellular location of MSP-1₁₉ polypeptides expressed by HEK-293 cells transfected with the recombinant plasmids. HEK-293 cells grown on cover slips were transfected with either P1 or P2 plasmid DNA constructs, cultured for 20 h, stained with mAb 5.2 followed by Alexa Flour 568-conjugated goat anti-mouse IgG. Cells were also counterstained with Hoechst 33258 for nuclei. In both panels, horizontal scale bar corresponded to 11.96 μm. (Upper Panel) Cells transfected with P1 construct and stained with Hoechst 33258 (A) or mAb 5.2 (B); (C) shows the merged image of (A) and (B). (Lower panel) Cells transfected with P2 construct and stained with Hoechst 33258 (D) or (E) mAb 5.2 (E); (F) shows the merged image of (D) and (E).

Figure 4.

Analysis of MSP-1₁₉-specific IgG levels in sera from mice immunized with the recombinant plasmids. BALB/c mice ($n = 5$ per group) immunized with P1 and P2 plasmid constructs and with control plasmid (Pv) were bled, sera prepared, and analyzed by ELISA using mAb 5.2. Antibody levels in each immunized mice at serum dilutions of 1:3000 (A) and 1:6000 (B) are shown. Both P1 and P2 immunized mice produced high antibody titers. P2 immunized mice showed significantly higher antibody levels than those immunized with P1.

Figure 5.

Evaluation of Ig isotypes in serum from mice immunized with the recombinant plasmids. Sera pooled from mice ($n = 5$) immunized with respective plasmids were analyzed for Ig isotypes by ELISA. The levels of MSP-1₁₉-specific IgG1, IgG2a, IgG2b, IgG3, IgM and IgA are shown. White and gray bars, respectively, represent mice immunized with P1 and P2. Statistical significance (p values) between Ig isotype responses produced by P1 and P2 are: 0.0814 for IgG1; 0.0176 for IgG2a; 0.0009 for IgG2b; 0.0564 for IgG3; 0.0636 for IgM; 0.1959 for IgA. * indicates significant difference.