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Malarial blood stage antigens can produce humoral immunity by induction of protective antibodies to epitopes on surface antigens on parasites. The merozoite surface protein-1 (MSP1) of *P. falciparum* is a major blood-stage vaccine candidate. MSP1 may play a role in binding and/or infection of erythrocytes by merozoites (parasites) during red blood cell invasion. The C-terminal fragment of MSP1, $MSP1_{19}$, is highly conserved among all known strains of *P. falciparum*. Immunization with recombinant proteins that have native-like conformations may elicit long-lasting protective immunity that mimics the natural immunity. We have used bacterial expression to tightly regulate transcription and translation of $MSP1_{42}$. Plasmids that encode $MSP1_{42}$ were prepared and tested for their ability to express recombinant protein. A three column purification scheme that included Ni^{+2} metal chelate chromatography, anion and then cation exchange chromatography, yielded greater than 95% pure $MSP1_{42}$. Immunoreactivity with mAbs showed that recombinant $MSP1_{42}$ was conformationally similar to native MSP1. The objectives were to develop recombinant $MSP1_{42}$ molecules that were structurally correct, (b) to develop fermentation and purification processes that could be scaled-up for large-scale processes, and ultimately (c) to advance these products into human clinical trials.

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INTRODUCTION

Over 300 million cases of malaria are reported each year worldwide, resulting in over 3 million deaths mostly among young children. The leading cause of clinical malaria is through infection with the parasite *Plasmodium falciparum*. Malaria caused by *P. falciparum* can lead to serious clinical illness and if untreated to death. Development of effective controls against malaria, either through the mosquito vectors or the parasite has lead to widespread pesticide and drug resistance, respectively. As these approaches have been unsuccessful, efforts have focused on malaria vaccine development.

Infection of *P. falciparum* through the mosquito vector initiates the complex life cycle within the human host. The parasite passes through progressive developmental stages that arise following invasion of the liver. Parasitic release from liver cells initiates the erythrocytic stage of the parasite life cycle. During the erythrocytic stage, red blood cells are invaded. Maturation and expansion of parasites, the merozoites during this stage, occurs within the red blood cells sequestered away from the host immune system. Vaccine candidates have been identified from each of the parasite's developmental stages. A leading erythrocytic stage candidate is the major merozoite surface protein, MSP1, [Diggs, *et al.*, 1993]. Vaccines derived

from malaria erythrocytic-stage antigens like MSP1 are of special interest because erythrocytic stages are the only confirmed targets of natural immunity among individuals from malaria endemic regions. Therefore, development of an efficacious erythrocytic stage vaccine from MSP1 or the C-terminal fragment ($MSP1_{42}$) has the potential to protect non-immune individuals. Since malaria-naïve individuals do not possess partial immunity developed through life-long exposures, immunization with $MSP1_{42}$ could induce the development of antibodies that are qualitatively comparable to those developed from natural malaria exposures. The mechanism of protection induced by an erythrocytic stage malaria vaccine would be mediated through the development of specific protective antibodies to proteins on the surface of parasites. Antibodies raised to parasite surface proteins would lead to an inability of the erythrocytic stage parasites (merozoites) to re-invade new erythrocytes. The suggested mode of action of these antibodies is to bind to the surface of the merozoites and block their ability to associate with, and invade erythrocytes, or to interfere with biochemical events associated with invasion. Blocking invasion would reduce the amplification of parasites in the bloodstream and thus reduce the overall parasitic load and severity of disease. Therefore, the development of specific antibodies to erythrocytic stage antigens like MSP1 could reduce

the likelihood of serious illness and disease in malaria-naïve individuals.

MSP1's function is not well understood [Holder and Blackman, 1994; Holder, *et al.*, 1988; Miller, *et al.*, 1993]. MSP1 is synthesized as a large 195-kDa precursor protein. It is anchored to the surface of the merozoite through a C-terminally attached N-glycosylphosphatidylinositol (GPI) anchor [Haldar, *et al.*, 1985]. Following release from red blood cells, proteolytic processing of full length MSP1 yields products with nominal molecular masses of 83, 28-30, 38-45 and 42 kDa. These processed polypeptides are recognized by MSP1 specific antibodies [Holder and Freeman, 1984; Lyon, *et al.*, 1986; Holder, *et al.*, 1987]. The MSP1 derived polypeptides remain noncovalently associated with each other at the surface of merozoites and are directly attached to the merozoite surface through the MSP1 42kDa C-terminal fragment [McBride and Heidrich, 1987; Lyon, *et al.*, 1987]. Either just before or during erythrocyte invasion, C-terminal $MSP1_{42}$ is processed again to a 33 kDa fragment and a 19 kDa C-terminal fragment ($MSP1_{19}$) [Blackman, *et al.*, 1991]. The non-covalently associated polypeptide complex is shed from the merozoite surface leaving only the 19 kDa fragment anchored through GPI. Following erythrocyte invasion, only $MSP1_{19}$ is present on ring forms in the newly invaded erythrocyte [Blackman, *et al.*, 1990].

The C-terminal $MSP1_{19}$ has a rather complex structure. Twelve cysteine residues occur within a span of 100 amino acid. Sequence alignment data suggests that two tandem domains form having homology at the level of cysteines with epidermal growth factor (EGF) [Blackman, *et al.*, 1991]. Each EGF-like domain contains six cysteine residues that could form three disulfide bridges per domain however, neither the number of disulfide bridges nor the linkage pattern of the disulfides has been directly confirmed on native MSP1.

Development of specific antibody responses to native MSP1 molecules requires that important conformational epitopes be present on the surface of these molecules. Several lines of evidence support the use of MSP1, and the C-terminal fragments, $MSP1_{42}$ and $MSP1_{19}$, as components of erythrocytic stage malaria vaccines. First, $MSP1_{19}$-specific monoclonal antibodies inhibit *P. falciparum* growth *in vitro,* [Blackman, *et al.*, 1990], or passively protect mice against infection with *P. yoelii,* [Majarian, *et al.*, 1984; Ling, *et al.*, 1994]. Second, immunization of monkeys with native MSP1, [Siddiqui, *et al.*, 1987], baculovirus-expressed recombinant $MSP1_{42}$ [Chang *et al*, 1996], or *S. cerevisiae*-secreted recombinant $MSP1_{19}$ (EVE-$MSP1_{19}$) from *P. falciparum* [Kumar, *et al.*, 1995], can protect against a homologous challenge. Similarly, *E. coli*-expressed recombinant $MSP1_{19}$ from *P. yoelii,* [Holder, *et al.*, 1994; Burns, *et al.*,

1989] protects against a homologous murine challenge. Finally, anti-sera raised against recombinant $MSP1_{42}$ [Chang, *et al.*, 1992], or $MSP1_{19}$ [Lyon and Haynes, unpublished] inhibit *P. falciparum* growth *in vitro*. The $MSP1_{19}$-specific monoclonal antibodies that either protect against infection *in vivo* [Burns, *et al.*, 1989], or inhibit parasite growth *in vitro* [Blackman, *et al.*, 1990], are specific for discontinuous epitopes since they do not react with disulfide-reduced $MSP1_{19}$ [McBride and Heidrich, 1987; Farley and Long, 1995]. Some monoclonal antibodies directed to $MSP1_{19}$ have been shown to inhibit parasite growth by blocking the proteolytic processing from 42kDa to 33kDa and 19kDa. A second class of monoclonal antibodies inhibits the ability of blocking antibodies and allows proteolytic processing to occur [Patino, *et al.*; 1997]. Therefore, the ability of antibodies to neutralize parasite development is dependent on the type of specificity developed to epitopes on the C-terminal $MSP1_{19}$. The development of antibodies with opposing specificity's may have arisen as a mechanism to avoid host immune responses. Thus, a recombinant vaccine product from this region of MSP1 may not only require correct disulfide-dependent conformation but selective development of specific antibodies to elicit a protective antibody response.

Successful expression of heterologous proteins in *E. coli* can lead to high levels of recombinant proteins. Bacterial

expression has the advantage of being relatively inexpensive, and is readily scaleable. Proteins expressed in bacteria are not post-translationally modified and heterologous proteins that require these modifications may have altered activities. Eukaryotic expression systems, such as yeast, baculovirus, or mammalian cells, post-translationally modify, however, the modifications may not be correct and protein yields can be poor. Induction of immune responses using heterologously expressed recombinant proteins requires that protein conformation and structure is correct. Heterologous expression of some recombinant MSP1 molecules ($MSP1_{42}$ and $MSP1_{19}$) from eukaryotic expression systems, *i.e.* baculovirus and yeast, have lead to recombinant proteins that are either correctly folded and expressed poorly or are mis-folded and expressed well, respectively. To avoid some of these issues, well-described bacterial expression systems (pET Expression System from Novagen) were used to express the C-terminal $MSP1_{42}$ fragment.

The objective of this work was to develop erythrocytic stage-specific malaria vaccine candidates that could elicit protective antibodies in volunteers in Phase I trials. Since MSP1 is a rather large protein, bacterial vaccine strategies have focused on proteolytic fragments. *In vitro* studies have shown that some protective epitopes on MSP1 may be contained within the C-terminal MSP1 cleavage product, $MSP1_{42}$. The C-

terminal $MSP1_{19}$ folds into a rather complex tertiary structure. Therefore, presentation of relevant epitopes on the C-terminal $MSP1_{19}$ is dependent on correct folding and conformation of $MSP1_{42}$. Recombinant plasmids were transformed into *E. coli* expression host, BL21(DE3) and recombinant proteins were expressed. Purification of recombinant $MSP1_{42}$ was through metal affinity chromatography using a six-histidine (His_6) amino acid sequence tag cloned at the N-terminus of the $MSP1_{42}$. Additional chromatography included anion exchange chromatography to remove endotoxin and nucleic acids and finally a cation exchanger, carboxymethyl resin, to concentrate the protein. Purified $MSP1_{42}$ will be formulated with an appropriate adjuvant and used in immunization studies of non-human primates and a human Phase I clinical trial to investigate the development of humoral immune responses.

The production of recombinant molecules using DNA technology and large-scale fermentation processes has enabled the development of proteins in quantities otherwise impossible. Although previously it was difficult to use bacteria to express some complex eukaryotic proteins, recent advances in the design of bacterial strains and the development of tightly regulatable expression vectors have lead to their use in expression of some complex eukaryotic proteins.

The construction of an $MSP1_{42}$ (3D7) expression vector to express and purify recombinant $MSP1_{42}$ proceeded through a series of vector constructions that ultimately led to the final clone, designated pET42AT(NK2), His_6-$MSP1_{42}$(3D7) (Figure 1). The final construct, so developed, was designed to meet the specifications required by the FDA for a product developed for human use from *E. coli*. The final His_6-$MSP1_{42}$ product contains a short N-terminal fusion on $MSP1_{42}$ that encodes six histidine residues and 11 linker amino acids. The plasmid also contains the gene for tetracycline selection. The detailed description of the construction of the DNA vector expressing a *P. falciparum* 3D7 $MSP1_{42}$ has been described elsewhere (Annual Report 1997).

Early stages of the process required optimization of fermentation and induction of expression of recombinant $MSP1_{42}$. A three hundred-liter scale GMP fermentation performed in June 1998 at Forest Glen Annex, Division of Biologics Research, provided adequate amounts of wet cell paste for purification process development. Optimal fermentation processes applied to large-scale fermentation can be empirically derived from small-scale culture conditions. Composition of the culture media, culture temperature, cell density at which induction of expression occurred, could have significant effects on the levels of protein produced and the total cell mass. Many heterologous proteins expressed in *E. coli* are either expressed

at very low levels, or insoluble in inclusion bodies. Expression of $MSP1_{42}$ was at good levels and the protein remained soluble when induction occurred at 25°C.

Affinity chromatography using Ni^{+2} chelating resins provides a simple, highly specific elution of desired target proteins. Six consecutive histidine residues are expressed as a short N-terminal fusion on the target protein. The application of cleared soluble supernatant fractions onto the Ni^{+2} agarose resin, allowed separation of other *E. coli* proteins from the recombinant His_6-$MSP1_{42}$. The His_6-$MSP1_{42}$ protein bound tightly through the imidazole ring in the histidine residues to the nickel ions immobilized by the NTA (nitrilotriacetic acid)(Qiagen) groups on the resin. Increasing concentrations of free imidazole compete with the His_6-$MSP1_{42}$ protein for binding to the matrix and result in elution of the tagged protein. Following this first chromatographic step, the $MSP1_{42}$ protein was present at greater than 60% purity. Additional chromatography was required to purify the protein to near homogeneity. Following extensive purification process development, $MSP1_{42}$ was purified to near homogeneity using two additional chromatographic steps; an anion exchanger, SuperQ 650M (TosoHaas), followed by a cation exchanger, CM650M (Tosohaas). The anion exchanger functioned to reduce the levels of endotoxin and nucleic acids present in the protein. The cation exchange

step removed a major *E.coli* contaminant and concentrated the protein. This 3-step chromatography yielded greater than 93% purity in $MSP1_{42}$.

During the course of the contract year, 1998, the $MSP1_{42}$ was scheduled for purification at the GMP scale on two separate occasions. In November, a 1.5kg wet cell paste was used to purify the $MSP1_{42}$ under the above-described conditions. The process was performed under nonGMP conditions, however, the process was performed at near GMP conditions, and with the exception, that formal documentation was not provided. This purification was performed to test the reproducibility of the purification process and to determine whether the process was scaleable. The SDS-PAGE and western blotting data from the November nonGMP purification showed that the protein was obtained at high levels of purity, however, the protein contained unacceptable levels of endotoxin (30EU/dose acceptable, November nonGMP, 60EU/dose). Endotoxin levels were determined both by *in vitro* gel clot LAL (*Limulus ameobocyte* lysate) and rabbit pyrogenicity tests. High levels of endotoxin suggest that the purification process was not sufficiently rigorous. Under optimal conditions, endotoxin is removed from aqueous solutions and some protein samples using anion exchange chromatography due to electrostatic attractions. In this case, although the endotoxin levels were not acceptable for animal

studies or human use, it was felt that with some modifications to the BPR (Batch Production Record), the endotoxin levels could be reduced to acceptable levels. Therefore, a second GMP purification scheduled for December would proceed. The changes made to the process were to increase the number of column volumes used to wash the Ni^{+2} column following the sample application. The data show that following this production, the level of protein purity was greater than 95% as measured by scanning densitometry (Figure 2 Coomassie Blue stained gel, western blots probed with mAb7F1 and mAb12.10). Immunoreactivity against $MSP1_{42}$ is with the major Coomassie blue stainable band and aggregated forms of $MSP1_{42}$. Gel clot LAL and rabbit pyrogenicity assays showed an even greater level of endotoxin, (600EU/dose). Since the endotoxin levels did not meet the release specifications for the final product, animal studies and the submission of the IND to the FDA was postponed pending re-evaluation of the purification process.

Work was initiated to re-develop the purification process of $MSP1_{42}$ with special attention to optimization of endotoxin removal at each chromatographic step. Review of the nonGMP and GMP purification BPR's and evaluation of an earlier to-scale purification performed in September 1998, showed that specific changes in buffer pH and detergent could lower endotoxin levels. Chromatography at pH 6.2 on Ni^{+2} metal chelate should result in

lower affinity of endotoxin for $MSP1_{42}$ and the column. At this pH the $MSP1_{42}$ closer to it's isoelectric point and has a reduced charge. The Ni^{+2} column would be weakly positively charged and endotoxin would easily pass through. Detergents, Tween 80, present in the buffers prior to cell lysis by microfluidization would reduce the interaction of endotoxin with proteins. In addition, Tween 80 was included in the dilution buffer and equilibration buffer for the anion exchanger, SuperQ. Detergents present during sample application may dissociate protein/endotoxin complexes. A last change was to lower the sodium chloride concentration during sample application on the SuperQ resin. This change increases the affinity of endotoxin binding to the SuperQ. These changes were introduced to the purification BPR and laboratory scale purification's showed that the levels of endotoxin were consistently within the acceptable range for human use when measured by gel clot and chromogenic LAL assays.

Production of low-endotoxin $MSP1_{42}$ began on April 12, 1999 at full scale (750g wet cell paste). Following the protein purification, the final product will be evaluated for endotoxin levels and induction of rabbit pyrogens. If endotoxin levels are in the acceptable range for this product, $MSP1_{42}$ will be tested for *in vivo* efficacy in nonhuman primate studies.

CONCLUSIONS

The presence of the native N-terminal sequences from $MSP1_{42}$ ($MSP1_{33}$) may have promoted proper folding and disulfide bond formation by initiating early productive folding pathways during protein translation. Expression of $MSP1_{42}$ (3D7) from the T7 promoter driven expression system avoided some problems previously found with bacterial expression of heterologous proteins, *i.e.* correct disulfide bond formation in the cytosolic reducing environment and partitioning of over-expressed recombinant heterologous proteins into insoluble inclusion bodies. The $MSP1_{42}$ protein was purified to near homogeneity following a three-column purification. Purified His_6-$MSP1_{42}$ protein was analyzed for identity and purity using SDS-PAGE and Coomassie Blue staining for total protein. Correct folding and disulfide bond formation of recombinant $MSP1_{42}$ was measured with a series of $MSP1_{19}$-specific conformation-dependent, reduction-sensitive mAbs and an $MSP1_{33}$-specific mAb on Western Blots (data not shown). These mAbs were developed against native parasite lysates and therefore they are relevant measures of native MSP1-conformation for specific epitopes on C-terminal MSP1. Future studies include evaluating $MSP1_{42}$ for its' ability to induce $MSP1_{42}$ specific antibodies and assessing *in vivo* efficacy in homologous challenge studies with *Aotus* monkeys.

REFERENCES

Blackman, M.J., Heidrich, H.G., Donachie, S., McBride, J.S. and Holder, A.A. (1990) A single fragment of a malaria merozoite surface protein remains on the parasite during red cell invasion and is the target of invasion-inhibition antibodies. J. Exp. Med. 172, 379-382.

Blackman, M.J., Whittle, H. and Holder, A.A. (1991) Processing of the *Plasmodium falciparum* major merozoite surface protein-1; Identification of a 33-kilodalton secondary processing product which is shed prior to erythrocyte invasion. Mol. Biochem. Parasitol. 49, 35-44.

Burns, J.M., Majarian, W.R., Young, J.F., Daly, T.M. and Long, C.A. (1989) A protective monoclonal antibody recognizes an epitope in the carboxyl-terminal cysteine-rich domain in the precursor of the major merozoite surface antigen of the rodent malarial parasite, *Plasmodium yoelii*. J. Immunol. 143, 2670-2676.

Chang, S.P., Gibson, H.L., Leeng, C.T., Barr, P.J. and Hui, G.S.N. (1992) A carboxyl-terminal fragment of *Plasmodium falciparum* gp 195 expressed by a recombinant baculovirus induces antibodies that completely inhibit parasite growth. J. Immunol. 148, 548-555.

Chang, S.P., Case, S.E., Gosnell, W.L., Kramer, K.J., Tam, L.Q., Hashiro, C.Q., Nikaido, C.M., Gibson, H.L., Leeng, C.T., Barr, P.J., Yokota, B.T., and Hui, G.S.N. (1996) A recombinant baculovirus 42-kilodalton c-terminal fragment of *Plasmodium falciparum* merozoite surface protein 1 protects *Aotus* monkeys against malaria. Infect. Immun. 64, 253-261.

Derman, A.I., Prinz, W.A., Belin, D., and Beckwith, J. (1993) Mutations that allow disulfide bond formation in the cytoplasm of Escherichia coli. Science. 262, 1744-1747.

Diggs, C.L., Ballou, W.R. and Miller, L.H. (1993) The major merozoite surface protein as malaria vaccine target. Parasitol. Today. 9, 300-302.

Farley, P.J. and Long, C.A. (1995) *Plasmodium yoelii yoelii* 17XL MSP-1: fine-specificity mapping of a discontinuous, disulfide-dependent epitope recognized by a protective

monoclonal antibody using expression PCR (E-PCR). Exp. Parasitol. 80, 328-332.

Haldar, K., Ferguson, M.A.J., and Cross, G.A.M. (1985) Acylation of a *Plasmodium falciparum* merozoite surface antigen via *sn*-1,2-diacylglycerol. J. Biol. Chem. 260, 4969-4974.

Holder, A.A. and Freeman, R.R. (1984) The three major antigens on the surface of *Plasmodium falciparum* merozoites are derived from a single high molecular weight precursor. J. Exp. Med. 160, 624-629.

Holder, A.A, Sandhu, J.S., Hillman, Y., Davey, L.S., Nicholls, S.C., Cooper, H. and Lockyer, M.J. (1987) Processing of the precursor to the major merozoite antigens of *Plasmodium falciparum*. Parasitology. 94, 199-208.

Holder, A.A. (1988) The precursor to major merozoite surface antigens: Structure and role in immunity. In: Malaria Immunology, Progress in Allergy. (Perlman, P. and Wigzell, K., eds.) pp. 72-97. Karger, Basel.

Holder, A.A, and Blackman, M.J. (1994) What is the function of MSP-1 on the malaria merozoite? Parasitol. Today. 10, 182-184.

Kumar, S., Yadava, A., Keister, D.B., Tian, J.H., Ohl, M., Perdue-Greenfield, K.A., Miller, L.H., and Kaslow, D.C. (1995) Immunogenicity and *in vivo* efficacy of recombinant *Plasmodium falciparum* merozoite surface protein-1 in *Aotus* monkeys. Mol. Med. 1, 325-332.

Ling, I.T., Ogun, S.A., and Holder, A.A. (1994) Immunization against malaria with a recombinant protein. Parasite Immunol. 16, 3-67.

Lyon, J.A., Geller, R.H., Haynes, J.D., Chulay, J.D. and Weber, J.L. (1986) Epitope map and processing scheme for the 195,000 dalton surface glycoprotein of *Plasmodium falciparum* merozoites deduced from cloned overlapping segments of the gene. Proc. Natl. Acad. Sci., USA. 83, 2989-2993.

Lyon, J.A., Haynes, J.D., Diggs, C.L., Chulay, J.D., Haidaris, C.G., and Pratt-Rossiter, J. (1987) Monoclonal antibody characterization of the 195-kilodalton major surface glycoprotein of *Plasmodium falciparum* malaria schizonts and merozoites: Identification of additional processed products and

a serotype-restricted repetitive epitope. J. Immunol. 138, 895-901.

Majarian, W.R., Daly, T.M., Weidanz, W.P., and Long, C.A. (1984) Passive protection against murine malaria with an IgG3 monoclonal antibody. J. Immunol. 132, 3131-3137.

McBride, J.S., Newbold, C.I., and Anand, R. (1985) Polymorphism of a high molecular weight schizont antigen of the human malaria parasite *Plasmodium falciparum*. J. Exp. Med. 161, 160-180.

McBride, J.S. and Heidrich, H.-G. (1987) Fragments of the polymorphic Mr 185,000 glycoprotein from the surface of isolated *Plasmodium falciparum* merozoites form an antigenic complex. Mol. Biochem. Parasitol. 23, 71-84.

Miller, L.H., Roberts, T., Shahabuddin, M., and McCutchan, T.F. (1993) Analysis of sequence diversity in the *Plasmodium falciparum* merozoite surface protein-1 (MSP-1). Mol. Biochem. Parasitol. 59, 1-14.

Patino Guevara, J.A., Holder, A.A., McBride, J.S., Blackman, M.J. (1997) Antibodies that inhibit malaria merozoite surface protein-1 processing and erythrocyte invasion are blocked by naturally acquired human antibodies. J. Exp. Med. 186, 1689-1699.

Siddiqui, W.A., Tam, L.Q., Kramer, K.J., Hui, G.S.N., Case, S.E., Yamaga, K.M., Chang, S.P., Chan, E.B.T., and Kan, S.-C. (1987) Merozoite surface coat precursor protein completely protects *Aotus* monkeys against *Plasmodium falciparum* malaria. Proc. Natl. Acad. Sci., USA. 84, 3014-3018.

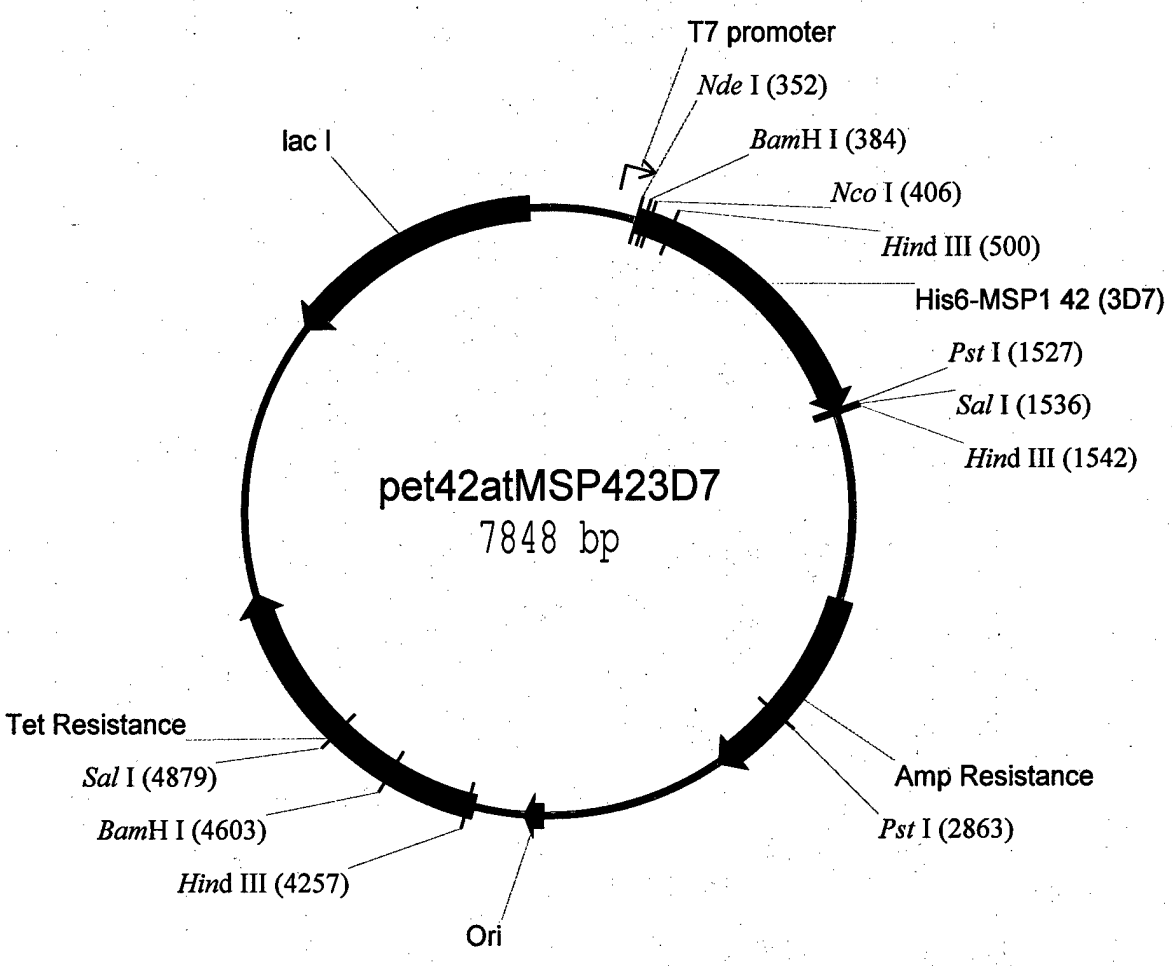
BIBLIOGRAPHY

1) ASBMB/ASIP/AAI Joint Meeting, New Orleans, June 1-6, 1996

Characterization Of A Recombinant *Plasmodium falciparum* MSP1 C-Terminal Fragment Using Conformation-Specific Monoclonal Antibodies. E. Angov[1], J. S. McBride[2], D. C. Kaslow[3], W.R. Ballou[1], C. L. Diggs[4], and J. A. Lyon[1]. [1]Immunol., WRAIR, Washington, D.C., 20307; [2]Univ. Edinburgh, U.K.; [3]NIAID, NIH, Bethesda, MD 20892; [4]USAID, Washington, D.C., 20523.

2) Miami Nature Biotechnology Symposium, Miami, February 1-5, 1997.

Structural Analysis of Refolded-Recombinant *Plasmodium falciparum* MSP1 C-Terminal Fragment By Using Conformation-Specific Monoclonal Antibodies. Evelina Angov[1], Jana S. McBride[2], David C. Kaslow[3], W.R. Ballou[1], Carter L. Diggs[4], and Jeffrey A. Lyon[1]. [1]Dept. Immunology, WRAIR, Washington, D.C., 20307; [2]Division of Biological Sciences, Univ. Edinburgh, EH9 3JT, U.K.; [3]NIAID, NIH, Bethesda, MD 20892; [4]USAIL, Washington, D.C., 20523.


T7 promoter
Nde I (352)
BamH I (384)
Nco I (406)
Hind III (500)
His6-MSP1 42 (3D7)
Pst I (1527)
Sal I (1536)
Hind III (1542)
lac I
pet42atMSP423D7
7848 bp
Tet Resistance
Sal I (4879)
BamH I (4603)
Hind III (4257)
Ori
Amp Resistance
Pst I (2863)

cGMP Purified, *E. coli* Expressed *P. falciparum* MSP1-42 (3D7)