ELSEVIER

Molecular and Biochemical Parasitology 85 (1997) 197–211

MOLECULAR AND BIOCHEMICAL PARASITOLOGY

# Antigenicity of recombinant proteins derived from *Plasmodium falciparum* merozoite surface protein 1

David R. Cavanagh *, Jana S. McBride

*Institute of Cell, Animal and Population Biology, Division of Biological Sciences, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh, EH9 3JT, UK*

Received 21 October 1996; accepted 23 December 1996

**Abstract**

We have expressed seven recombinant antigens representing two N-terminal regions of the polymorphic merozoite surface protein 1 (MSP-1) of *Plasmodium falciparum*. The antigens include the MAD20 and Palo Alto forms of the relatively conserved Block 1 region, and variants of the Block 2 region from isolates 3D7, Palo Alto FUP, MAD20, Wellcome and RO33, that are representative of a range of amino acid sequence diversity in this most polymorphic section of MSP-1. All recombinant antigens have been able to immunise mice to produce polyclonal antibodies which specifically recognise parasite MSP-1 in indirect immunofluorescence assays and in Western blots. The recombinant antigens also react appropriately in ELISA with murine monoclonal antibodies specific for variant epitopes in Block 2 of MSP-1. These results show that the antigenic structure of the recombinant proteins is similar to that of the native MSP-1 product from parasites. Importantly, human sera from malaria-exposed individuals contain IgG antibodies that recognise very specifically one or another of the Block 2 types, showing that different Block 2 types are immunogenic, antigenically distinct and distinguishable when presented during natural infections. In contrast, the conserved Block 1 is rarely recognised by human antibodies. © 1997 Elsevier Science B.V.

*Keywords:* Malaria; *Plasmodium falciparum*; Antibody; Recombinant antigens

## 1. Introduction

*Plasmodium falciparum* is the major causative agent of human malaria, responsible for several hundred million clinical cases and 1–2 million deaths per year. Antigens of the invasive blood stage of the parasite, the merozoite, have been proposed as vaccine targets [1]. The major con-

*Abbreviations:* GST, gluthathione-*S*-transferase; IFA, immunofluorescence assay; MSP-1, merozoite surface protein 1; PCR, polymerase chain reaction.

* Corresponding author. Tel.: +44 131 6505459; fax: +44 131 6673210; e-mail: david.cavanagh@ed.ac.uk


*PII* S0166-6851(96)02826-5

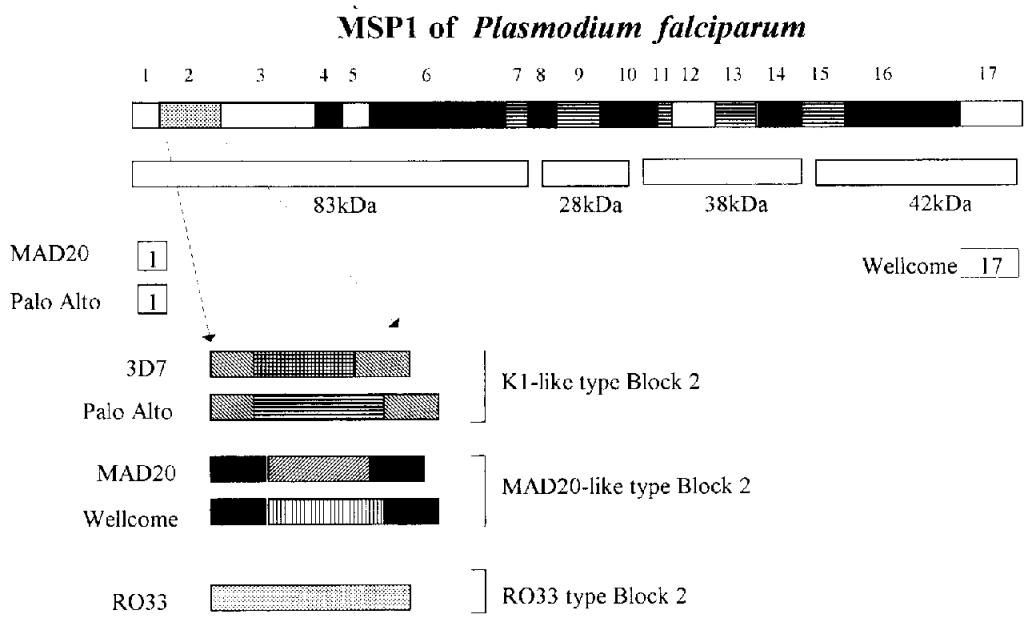


Fig. 1. Schematic representation of MSP-1 of *P. falciparum* and of recombinant MSP-1 antigens. The division into 17 blocks is as outlined by Tanabe et al. [9]; blocks of conserved sequences are denoted by open boxes; regions of dimorphic or semi-conserved sequences are denoted by full or hatched boxes respectively, and the polymorphic Block 2 region is shown as a speckled box. The scheme of natural processing of MSP-1, shown below the full length protein, is after Holder et al. [47]. Recombinant forms of Block 1 and Block 17 (on scale) and of Block 2 variants (enlarged) derived from MSP-1 alleles of the indicated *P. falciparum* isolates are shown below the main diagram.

stituents of the merozoite surface are polypeptides derived from a high molecular mass precursor protein, merozoite surface protein 1 (MSP-1, also known as p190, gp195, PMMSA and MSA1). This protein is synthesised during schizogony and its products are localised on the surface of extracellular merozoites [2,3]. Post-translational proteolytic processing of the precursor molecule generates fragments of 83, 42, 38, and 28–30 kDa which persist as a non-covalently linked complex on the surface of mature extracellular merozoites [3–5]. Subsequent processing of the 42 kDa fragment to produce the 19 kDa C-terminal fragment occurs at the time of red cell invasion [6]. Only the 19 kDa fragment is retained on the surface of merozoites throughout invasion of erythrocytes, all other fragments being shed before or at this event [6–8].

Comparison of nucleotide sequences of MSP-1 genes from different field and laboratory isolates of *P. falciparum* has shown that the amino acid sequence of the protein is variable and can be divided into 17 distinct blocks defined by their content of conserved, semi-conserved or variable sequences [9, reviewed in 10, Fig. 1]. Variants of *P. falciparum* MSP-1 can be grouped by amino acid sequence homologies into two distinct serogroups [11,12] corresponding to two major allelic families represented by variants of the MAD20 and Wellcome isolates [9,10]. Although this sequence dimorphism is true of the molecule as a whole, one exception is a polymorphic region, Block 2, where extensive sequence diversity exists. Over 50 different sequences have been identified in Block 2, which fall into three main types represented by variants of the K1, MAD20 and RO33 isolates. Block 2 sequences of the K1-like and MAD20-like types contain variable tri- or hexa- peptide repeats, whereas Block 2 of the RO33 type contains a non-repetitive sequence which varies only little.

Several studies have shown that immunisation with MSP-1 derived from *P. falciparum* can be used to protect monkeys from experimental infec-

tion [13–19]. *Aotus* monkeys immunised with parasite-derived Palo Alto variant of MSP-1 were completely protected from the lethal effects of challenge with the same parasite [13], whereas only partial protection from lethal infection was achieved after immunisation with purified K1 parasite MSP-1, when challenged with non-homologous parasites [14]. Other studies showed that immunisation with parasite-derived MSP-1 of the K1 variant could protect *Saimiri* monkeys from lethal challenge with the non-homologous Palo Alto variant of the parasite [15]. The reasons for the difference in efficacy of heterologous immunogens in the two studies were not clear, but were attributed partly to the high degree of homology between the N-terminal regions of the two MSP-1 variants used in the latter study [15]. Similarly, *Aotus* and *Saimiri* monkeys immunised with recombinant MSP-1 fragments were shown to be partially protected from experimental challenge [15–19]. Thus, immunisation with proteins derived from MSP-1 has been shown to protect animals from experimental infection, indicating its usefulness as a potential vaccine target.

It is not known which part(s) of the protein contain epitopes important in protective immunity induced by the whole native protein. Much interest has focused on one conserved region, $MSP\text{-}1_{19}$ [reviewed in [20]], while other regions have remained relatively neglected. Two regions from the N-terminal end of MSP-1, the semi-conserved Block 1 and the highly polymorphic Block 2, deserve attention. Block 1 contains the semi-conserved amino acid sequence (YSLFQKEK-MVL) that was included in the Spf66 vaccine tested in clinical and field trials [21–23]. Recently, a monoclonal antibody, specific for a variant epitope in the Block 2 region, has been shown to inhibit parasite growth in vitro [24]. Field seroepidemiology studies on human antibody responses to MSP-1 delivered by natural infections in Africa, have suggested that polymorphic and dimorphic sequences in the molecule may play a major role in inducing protective immunity [25,26]. Increased levels of IgG against an N-terminal fragment of MSP-1 were found in subjects from a mesoendemic area in Gabon who had cleared infections compared with those who had persistent infection [27]. Other studies suggested that the lower the level and the shorter-lived the humoral response to N-terminal regions of MSP-1, the higher the risk of subsequent reinfection [26]. In contrast, one study indicated that antibodies to several regions of the MSP-1 molecule, including the polymorphic Block 2 region, are correlated with increased risk of reinfection and/or decreased ability to control parasitaemia, but it was suggested that this might reflect a history of numerous infections and therefore reveal individuals who were more susceptible to infection [28].

To elucidate the effect of allelic variation on antigenic polymorphism and on the specificity of immune responses to two N-terminal regions of MSP-1, here we evaluate the antigenicity of new recombinant proteins representing the extreme N-terminal Block 1 which is relatively conserved and all three main types of the polymorphic Block 2 region. Mouse antisera raised against the recombinant antigens and monoclonal antibodies to various regions of MSP-1, were used to show that the Block 1 and Block 2 antigens possess conserved and type-specific epitopes respectively, of the native protein. Importantly, serum Abs from malaria exposed individuals recognised and distinguished these type-specific epitopes, showing that the polymorphic Block 2 region is immunogenic when delivered to the human immune system during natural *P. falciparum* infections.

## 2. Materials and methods

### 2.1. PCR amplification of MSP-1 gene fragments

DNA was obtained from the *P. falciparum* clone 3D7 [29] and isolates Palo Alto FUP [30], MAD20 [9] Wellcome [7] and RO33 [31], maintained by the WHO Registry of Standard Strains of Malaria Parasites at the University of Edinburgh. Parasites were grown in vitro by previously described methods [32,33]. Parasite DNA was purified using the method of Fenton et al. [34] and used as a template for fragment-specific polymerase chain reactions (PCR). Each reaction contained 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin, 2 mM each

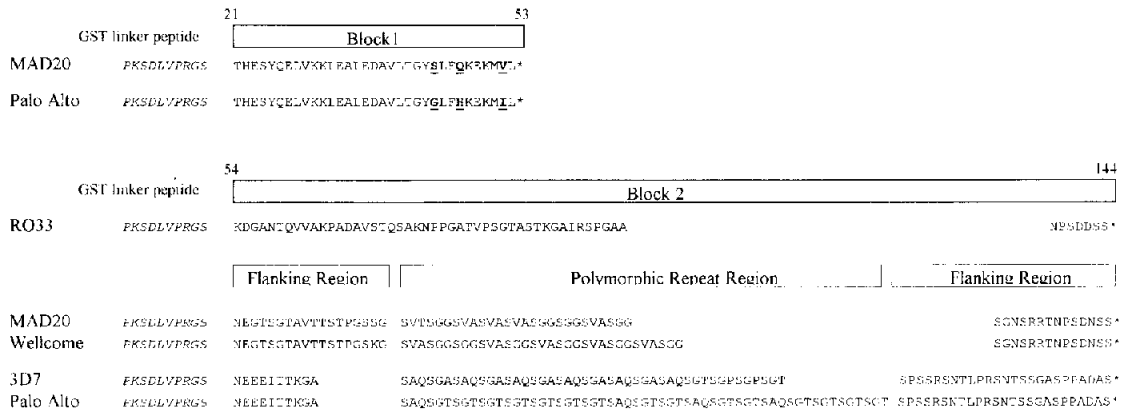


Fig. 2. Sequences of the recombinant MSP-1 Block 1 and Block 2 fusion proteins developed in this study. Each sequence is shown in the one letter amino acid format. Numbering of MSP-1 sequence as in Miller et al. [10]. Block 1 includes residues 21–53; Block 2 variants include residues 54–144.

dNTP, 2.5 units Taq DNA polymerase (Bochringer-Mannheim) and 1 mM each of the following pairs of primers: forward primer (5′ CTGGATCCAATGAAGAAGAAATTACT 3′) and reverse primer (5′ GGGAATTCTTAGCTTGCATCAGCTGGAGG 3′) were used to amplify 3D7 and Palo Alto Block 2 regions; forward primer (5′ CTGGATCCAATGAAGGAACAAGTGGA 3′) and reverse primer (5′ GGGAATTCTTAACTTGAATTATCTGAAGG 3′) for Block 2 regions of MAD20 and Wellcome; forward primer (5′ CTGGATCCAAGGATGGAGCAAATACT 3′) and reverse primer (5′ GGGAATTCTTAACTTGAATCATCTGAAGG 3′) for the RO33 Block 2 region; forward primer (5′ CCCGGATCCGTAACACATGAAAGT TAT 3′) and reverse primer (5′ CCCGAATTCTTATAA[A/C]A[T/C]CATTTTTTCCTT 3′) for Palo Alto FUP and MAD20 Block 1 regions. Underlined portions of each primer indicate non-MSP-1 sequences added to incorporate *Bam*HI and *Eco*RI restriction enzyme sites into the PCR products, whereas non-underlined parts of these primers match specifically the 5′ and 3′ ends of the three main types of MSP-1 Block 2 sequences, or the two main types of MSP-1 Block 1 sequences [7,9,30,31]. A polymerase chain reaction (PCR) cycle of 95°C, 90 s; 50°C, 15 s; 72°C, 45 s was repeated for 35–40 cycles in each case.

### 2.2. Production of recombinant MSP-1 antigens

PCR amplified fragments of the MSP-1 gene were expressed in *Escherichia coli* as recombinant proteins fused to the C-terminus of glutathione *S*-transferase (GST) of *Schistosoma japonicum* using the pGEX-2T vector [35].

A single DNA fragment was amplified by PCR from each sample of genomic DNA, purified from agarose gels using Prepagene (Bio-Rad) according to the manufacturer's instructions, digested with 15 units each of *Eco*R I and *Bam*HI restriction endonucleases (Boehringer Mannheim) and ligated with *Eco*RI and *Bam*HI cut pGEX-2T plasmid DNA. Ligation mixes were used to transform *E. coli* strain DH5α. Transformants carrying recombinant plasmids with DNA inserts were screened for expression of fusion proteins 3.5–7.5 kDa larger than GST, depending on the MSP-1 DNA insert in question. The sequence and orientation of the MSP-1 inserts in each construct was determined by DNA sequencing [36]. Fig. 2 shows the deduced amino acid sequences of Block 1 and Block 2 recombinant proteins reported in this study. Each GST fusion protein was produced and purified in bulk from 500 ml cultures of *E. coli* harbouring the appropriate GST-MSP-1 plasmid construct. The GST-fusion proteins were purified to homogeneity by affinity chromatography on glutathione agarose beads (Sigma). Con-

trol antigen, GST, was purified from *E. coli* harbouring the pGEX-2T vector alone. Aliquoted proteins were stored in elution buffer (50 mM Tris pH 8.0, 10 mM reduced glutathione) at −70°C until needed.

A pGEX construct which encodes a GST fusion protein containing most of the 19 kDa C-terminal fragment of MSP-1 (MSP-$1_{19}$) was kindly provided by Dr A. Holder of the NIMR, Mill Hill, London, UK. It contains the C-terminal fragment from $Asn_{1631}$ to $Asn_{1726}$ of the Wellcome isolate [37].

### 2.3. Immunisations

Purified GST fusion proteins were used as immunogens to produce polyclonal antisera in CBA/Ca and MF1 strains of mice. Immunising antigen was prepared by diluting each purified fusion protein in phosphate buffer saline (PBS) (7.15 mM $Na_2HPO_4$, 2.85 mM $KH_2PO_4$, 3.58 mM KCl, 0.134 M NaCl), adding 4 volumes of adjuvant (Imject Alum, Pierce) dropwise and stirring for 30 min. This mixture was used to immunise mice intraperitoneally. For each mouse, 50 μg of protein in a final volume of 400 μl was used for each immunising dose. Three doses were given at monthly intervals and blood was collected 12–13 days after each boosting.

### 2.4. MSP-1-specific monoclonal antibodies and human sera

Monoclonal antibodies used for analysis by ELISA of the recombinant antigens reported here are described in the legend to Fig. 4. Sera from 43 patients (aged 1–34 years) presenting with uncomplicated *P. falciparum* malaria to the Outpatients Department of the Medical Research Council at Fajara, The Gambia, West Africa, were collected during malaria transmission seasons (October to December) in 1982 and 1983. A sample of 0.3 ml of blood was obtained from each patient as part of a venous sample for other studies [11], after consent, under the approval of the Scientific and Ethical Committees of the Medical Research Council and of the Gambian government and sera were stored at −70°C. Control sera of Europeans who had not been exposed to malaria infection were from 37 healthy adult donors to the Scottish Blood Transfusion Service.

### 2.5. Indirect immunofluorescence assays (IFA)

Specificities of mouse polyclonal antisera made to the recombinant MSP-1 proteins for a selection of *P. falciparum* isolates expressing different MSP-1 alleles were compared by indirect immunofluorescence assays (IFA) as described [12,38]. Serial dilutions of the antisera (1:50–1:1600) or control monoclonal antibodies, were made in PBS containing 1% bovine serum albumin (BSA) and 0.01% sodium azide. A 25 μl volume of each antiserum was incubated on a well of a multispot slide (Hendley, Essex, UK) with acetone-fixed schizonts at room temperature for 30 min, followed by washing of the slides in PBS (1 min/5 min/5 min). The slides were dried for 10 min at 50°C, then each spot was incubated with 15 μl of 1:80 dilution of FITC (fluorescein isothiocyanate) conjugated rabbit anti-mouse Ig (ICN Immunobiologicals) for 30 min at room temperature. After two washes in PBS, the slides were immersed in a solution of 0.1% (w/v) Evans Blue and 0.001% (w/v) DAPI (4′,6-diamino-2-phenylindole, Sigma) in PBS for 5 min to counterstain erythrocytes and parasite nuclei respectively. The slides were washed and mounted in Citifluor (City University, London) under cover-slips. The parasites were visualised by DAPI-fluorescence (DNA specific) and antibody-reactive parasites by FITC-fluorescence (Ab specific) with incident light of 450–490 nm and 390–440 nm respectively, at a magnification of ×600.

### 2.6. Western blotting

Schizont proteins extracted in SDS-sample buffer (50 mM Tris pH 6.8, 5% [v/v] 2-mercaptoethanol, 2% [w/v] SDS, 0.1% [w/v] bromophenol blue, 10% [v/v] glycerol) were resolved by discontinuous SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in 8% acrylamide gels and transferred to Protran BA85 membranes (Schleicher and Schuell) in a Transfor electrophoresis unit (Hoefer, UK) at a constant cur-

rent of 70 mA with cooling for 3 h. The membranes were incubated in blocking buffer (5% non-fat milk powder in PBS, supplemented with 0.05% Tween 20 and 0.02% $NaN_3$) for 1 h and the transferred proteins then probed for 3 h with polyclonal mouse sera against the recombinant proteins diluted 1:50–1:400 in blocking buffer. The membranes were washed three times for 10 min each in washing buffer (0.1% (v/v) Tween 20 in PBS), incubated with horseradish peroxidase-conjugated rabbit anti-mouse IgG antibody (Dako, UK) diluted 1:400 in washing buffer for 1 h, washed three times as above and finally rinsed in 10 mM Tris, 0.9% NaCl, pH 7.4. Binding of mouse IgG to schizont proteins was visualised using $H_2O_2$ and 4-chloro-1-naphthol as the chromogenic substrate [39].

### *2.7. Enzyme-linked immunosorbent assay (ELISA)*

Human sera and murine monoclonal antibodies were tested by ELISA for their ability to recognise the recombinant MSP-1 fragments. Wells of 96-well plates (Immulon 4, Dynatech) were coated with 50 ng/well of recombinant antigens in 100 $\mu$l of coating buffer (15mM $Na_2CO_3$, 35mM $NaHCO_3$, pH 9.3) overnight at 4°C. The wells were washed three times in washing buffer (0.05% Tween 20 in PBS). Unoccupied protein binding sites were blocked with 200 $\mu$l/well blocking buffer (1% [w/v] skimmed milk powder in washing buffer) for 5 h at room temperature and washed again three times. Mouse mAbs or human sera diluted in the blocking buffer (100 $\mu$l per well) were added to duplicate antigen-coated wells overnight at 4°C. After three washes, the wells were incubated for 3 h with 100 $\mu$l per well of horseradish peroxidase (HRP)-conjugated polyclonal rabbit anti-mouse IgG (at 1:1000) or rabbit anti-human IgG (at 1:5000) (Dako Ltd., High Wycombe, UK) and washed three times before incubating for up to 15 min at room temperature with 100 $\mu$l of substrate (0.1 mg $ml^{-1}$ *O*-phenylenediamine [Sigma] and 0.012% $H_2O_2$) in development buffer (24.5 mM citric acid monohydrate and 52 mM $Na_2HPO_4$, pH 5.0). The reaction was stopped with 20 $\mu$l of 2M $H_2SO_4$ and absorbance was measured at 492 nm. The cut-off level at which antibody binding from malaria exposed individuals to any one antigen, was regarded as significantly above background that was calculated as the mean plus two standard deviations of absorbance readings obtained with sera from 37 Scottish blood donors with no history of exposure to malaria.

Competition for human antibody between pairs of antigens was also measured by ELISA. Aliquots (100 $\mu$l) of selected sera diluted 1:500 were first reacted with 0–10 $\mu$g $ml^{-1}$ of soluble competing antigen i.e. with up to 20-fold excess of 50 ng plate-bound antigen, then placed on the plate-bound antigen in wells overnight. This was followed by incubation with HRP-conjugated second antibody and the rest of the procedure as described above.

## 3. Results

### *3.1. Expression of MSP-1 Block 1 and Block 2 sequences as recombinant proteins*

All but one of the MSP-1 sequences cloned in pGEX-2T (Fig. 2) were found to be identical to published sequences [7,9,30,31]. DNA sequencing of the Block 2 region from the 3D7 clone of *P. falciparum* revealed differences from the published sequence [40]. The deduced amino acid sequence of the 3D7 recombinant protein contains six GASAQS hexapeptide repeats, not five as previously reported, and is more closely related to the MSP-1 sequence of isolate NF7 [41] (Fig. 2). The original authors [40] have recently submitted this corrected sequence of 3D7 to Genbank (Acc. No. X52963).

All MSP-1 recombinant fusion proteins were produced in a soluble form, and were purified to homogeneity by affinity chromatography on glutathione agarose beads. Protein yields varied between 4 mg $l^{-1}$ of *E. coli* culture for Block 1 constructs to 30 mg $l^{-1}$ for Block 2 constructs.

### 3.2. Differentiation of MSP-1 allelic variants by antisera raised against recombinant proteins

Antisera produced in mice against Block 1 and Block 2 recombinant fusion proteins were tested by IFA and Western blotting for specificity of reactivity with schizonts expressing known allelic variants of MSP-1. IFA reactivities of antisera raised against the five variant Block 2 antigens are summarised in Table 1 and immunoblot reactivities of all antisera with schizont proteins are shown in Fig. 3.

In IFA, sera of 20/20 mice immunised against either the Palo Alto or MAD20 variants of Block 1 recognised equally strongly schizonts of all isolates tested, with no differentiation between parasites expressing either the MAD20 or Palo Alto variant of Block 1 (data not shown). Seventeen out of 18 positive sera raised against either the 3D7 or Palo Alto recombinant Block 2 antigens specifically recognised schizont and merozoite stages of both these isolates, but most were unable to differentiate well between these isolates that both express a K1-like type of Block 2, despite each antigen containing different internal repetitive sequences (Fig. 2; Table 1, columns 3D7 and Palo Alto). However, these sera were all highly specific for the K1-like type of Block 2 as they failed to react with any parasites expressing the other two types of Block 2 (MAD20 and Wellcome, or RO33). Specificity for the MAD20-like type but lack of differentiation within this type was also shown by all 18 positive antisera raised against the MAD20 or Wellcome recombinant Block 2 antigens, that contain non-identical repeat sequences (Fig. 2; Table 1, columns MAD20 and Wellcome). Ten sera raised against the recombinant RO33 Block 2 antigen reacted specifically with RO33-type parasites (Table 1, column RO33), but not with any parasites of the K1 or MAD20 types.

Western blots of schizont proteins probed with the same antisera showed the same specificity as IFA for different variants of MSP-1 (Fig. 3). Thus sera against recombinant Block 1 proteins recognised all variants of MSP-1 tested (including intermediate processing products, Fig. 3, Panel A). Antisera to recombinant K1-like Block 2 types reacted specifically with 3D7 and Palo Alto native MSP-1 proteins, but not with MAD20-like or RO33 variants expressed by the other tested parasites (Fig. 3, Panels B and C). Sera raised against recombinant MAD20-like Block 2 types reacted solely with MAD20-type parasites' MSP-1 products, including the N-terminal MSP-183 fragment (Fig. 3, Panels D and E). Finally, sera against the recombinant RO33 Block 2 protein recognised only the MSP-1 products of RO33 type parasites (Fig. 3, panel F). These highly specific reactions in Western blots with schizont MSP-1 confirmed and extended the results of the IFA experiments, and showed that the recombinant Block 2 antigens are antigenically similar to native MSP-1 and can induce antibodies which recognise parasite MSP-1 in type-specific manner.

### 3.3. Reactivity of recombinant Block 2 antigens with murine monoclonal antibodies

Murine mAbs specific for various domains of the MSP-1 molecule were used to probe further the antigenic integrity of the recombinant Block 1 and 2 antigens by ELISA (Fig. 4; legend lists the domain- and type-specificities of the mAbs). No mAbs specific for the Block 1 region, or the MAD20 and Wellcome variants of Block 2, were available and these four antigens tested negative with all antibody reagents. Other Block 2 fusion proteins were specifically recognised only by mAbs directed against that region of MSP-1. Monoclonal antibodies 12.3D3.10 and CE2.1, which are highly specific for (SGT)3 and (SGT)5 repeats typical of the Palo Alto isolate, reacted solely with the Palo Alto Block 2 fusion protein. The mAb 12.2-1-1 which recognises the 3D7 repeats GASAQS and similar K1-like Block 2 sequences, reacted with both 3D7 and Palo Alto recombinant Block 2 antigens containing the target sequence. Three RO33-specific mAbs (31.1, 31.2 and 31.7) reacted solely with the recombinant RO33 Block 2 antigen. Monoclonals 12.10-5, 12.8-2, 2.2-7 and 5.2, specific for the C-terminal MSP-1 fragment, were included as negative controls in the assessments of Block 2 antigens. These mAbs, all specific for disulphide-dependent epitopes of native MSP-$1_{19}$, reacted only with the

Table 1
Immunofluorescence reactions of mouse sera to recombinant Block 2 proteins with schizonts of *P. falciparum* isolates

| Immunogen | Mouse strain immunised | Mouse number | Titre | Parasite isolate tested | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 3D7 | Palo Alto | MAD20 | Wellcome | RO33 |
| 3D7 Block 2 | CBA/Ca | 1 | 50 | ++ | ++ | − | − | − |
| | | 2 | 50 | ++ | ++ | ± | ± | ± |
| | | 3 | 50 | ++ | ++ | − | − | − |
| | | 4 | 50 | ++− | +++ | ± | − | ± |
| | | 5 | 100 | ++− | +++ | ± | | |
| | MF1 | 1 | 1000 | +−+ | + | − | − | − |
| | | 2 | 50 | ++−+ | +++ | ± | − | ± |
| | | 3 | 400 | ++−+ | + | − | ± | − |
| | | 4 | 100 | ++++ | −+++ | − | − | − |
| Palo Alto Block 2 | CBA/Ca | 1 | 50 | + | + | − | − | − |
| | | 2 | 50 | − | − | ± | ± | − |
| | | 3 | 100 | +++ | −++ | ± | − | − |
| | | 4 | 150 | ++ | −+ | − | − | − |
| | | 5 | 50 | + | −+ | − | − | − |
| | MF1 | 1 | 1000 | +++ | −+++ | − | − | − |
| | | 2 | 1000 | +++ | −+++ | − | − | − |
| | | 3 | 1000 | ++ | −+++ | − | − | − |
| | | 4 | 1000 | + | −+++ | − | − | − |
| | | 5 | 500 | ± | −+++ | − | − | − |
| MAD20 Block 2 | CBA/Ca | 1 | 100 | − | − | ++ | ++ | − |
| | | 2 | 400 | − | − | ++ | ++ | − |
| | | 3 | 100 | − | − | ++++ | +++ | − |
| | | 4 | 50 | − | − | ++ | ++ | − |
| | MF1 | 1 | 50 | | | ++ | −+ | − |
| | | 2 | 400 | − | − | ++++ | −++ | − |
| | | 3 | 400 | − | ± | ++++ | −+ | − |
| | | 4 | 200 | − | − | ++++ | −++ | − |
| | | 5 | 400 | − | − | +++ | −+ | − |
| Wellcome Block 2 | CBA/Ca | 1 | 50 | − | − | + | + | − |
| | | 2 | 50 | − | ± | + | + | |
| | | 3 | 50 | ± | − | ++ | −+ | − |
| | | 4 | 50 | ± | − | + | −+ | − |
| | | 5 | 50 | − | − | ++++ | −++ | − |
| | MF1 | 1 | 50 | | − | + | + | − |
| | | 2 | 100 | − | − | ++ | −+ | − |
| | | 3 | 50 | − | − | − | − | |
| | | 4 | 50 | − | − | + | + | − |
| | | 5 | 100 | + | − | ++− | −+ | ± |
| RO33 Block 2 | CBA/Ca | 1 | 100 | − | | − | − | −++ |
| | | 2 | 100 | − | − | ± | ± | +++ |
| | | 3 | 600 | − | − | | − | ++ |
| | | 4 | 800 | − | ± | − | | +++ |
| | | 5 | 800 | − | − | − | − | +++ |
| | MF1 | 1 | 1000 | − | − | − | − | +++ |
| | | 2 | 3000 | − | − | − | − | +++ |
| | | 3 | 100 | − | − | − | − | +++ |
| | | 4 | 50 | | − | − | − | +++ |
| | | 5 | 1000 | | − | − | − | +++ |

Recombinant immunogens and mouse strains are shown in columns 1 and 2 respectively. The table shows responses of individual mice on day 13 after secondary immunisation. Sera were first titrated between 1:50 and 1:1600 on homologous parasites for the highest dilution at which a strong reaction could still be seen, and were then tested at that dilution on other isolates that differ in Block 2 of MSP-1 [10]. The relative strength of the reactivity of each serum with schizont stage parasites from five parasite types tested is shown: (++++) very bright schizont-specific fluorescence (+++), bright schizont-specific fluorescence; (++), easily visible schizont-specific fluorescence; (+), visible schizont-specific fluorescence; (±), inconsistent fluorescence; (−), no fluorescence.

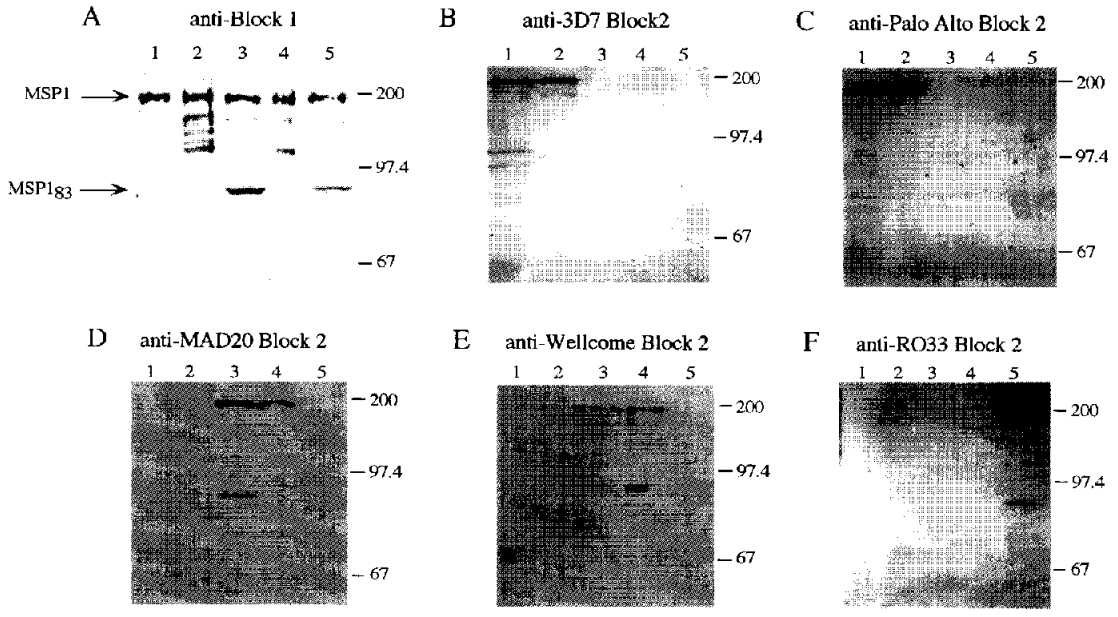


Fig. 3. Specific reactions of schizont MSP-1 and its natural processing product MSP-$1_{83}$ with antisera made against recombinant Block 1 or Block 2 fusion proteins. Pooled sera produced by groups of mice immunised with indicated recombinant antigens were used to probe Western blots of schizont proteins run on 8% SDS-PAGE gels. Total schizont proteins of five *P. falciparum* isolates were probed as follows: lane 1 Palo Alto FUP; lane 2 3D7; lane 3 MAD20; lane 4 Wellcome; lane 5 RO33. Panel A, anti-Block 1 serum; B, anti-Block 2 Palo Alto; C, anti-Block 2 3D7; D, anti-Block 2 MAD20; E, anti-Block 2 Wellcome; F, anti-Block 2 RO33. The positions of molecular weight markers run in parallel with the schizont extracts are shown for each blot. The position of MSP-1 and its N-terminal processing fragment MSP-183 (present only in extracts of parasites in lanes 3 and 5) are marked in panel A; other intermediate processing products of MSP-1 are present in lanes 2 and 4.

recombinant MSP-$1_{19}$. Other mAbs were specific for epitopes within regions of the MSP-1 molecule outside those contained in the recombinant antigens, which failed to react with these negative control mAbs as expected.

### 3.4. Reactivity of recombinant MSP-1 antigens with human IgG antibodies

Antibody recognition of the panel of recombinant MSP-1 antigens including all variants of Block 1, Block 2 and MSP-$1_{19}$, was tested by ELISA against 43 sera from malaria-exposed donors (1–34 years old) from The Gambia and also against 37 sera from non-exposed adult Europeans as controls. Results are shown in Figs. 5 and 6. All seven antigens tested were specifically recognised by IgG serum antibodies from some malaria-exposed individuals but not by non-exposed controls. Only one malaria-exposed individual (0.2%) had antibodies to the relatively conserved Block 1 region, whereas 32 (75%) individuals had antibodies which recognised the conserved MSP-$1_{19}$ antigen. Variable numbers of individuals recognised the Block 2 antigens, from 8 (19%) for the 3D7 antigen to 16 (37%) for the Palo Alto antigen. In all cases, IgG antibodies from malaria-exposed donors differentiated clearly between the K1-like, MAD20-like and RO33 types of Block 2, but failed to differentiate between variants within a type (typical examples shown in Fig. 6). For example, antibodies from donor W3 recognised both K1-type Block 2 antigens (3D7 and Palo Alto). Similarly, antibodies from donor F8 recognised both tested MAD20-type variants (MAD20 and Wellcome), and to a lesser extent the K1-type Block 2 antigens. This is explained by the presence of both K1-like and MAD20-like parasites in the blood of this individual as detected by PCR (data not shown). RO33

Block 2-specific responses (donor GF20, Fig. 6) are also type specific and show no cross-reaction with other Block 2 types.

To analyse in detail specificities of the sera for variants of Block 2, competitive inhibition ELISAs were performed using pairs of similar recombinant antigens, one as the capture antigen on the plate to detect IgG binding, the other as a competing antigen added in increasing amounts to serum. Sera W3 (K1-type recognition) and F8 (MAD20-type recognition) were tested with pairs of antigens within these types (Fig. 7).

For serum W3, there was little difference in the capacity of the two tested K1-type variants (3D7 or Palo Alto) to compete with each other for antibodies, indicating this serum contained antibodies that cross-reacted with both recombinant antigens. In reciprocal experiments, complete inhibition of antibody binding to either capture antigen was achieved with approximately the same excess of competing antigen, indicating that this serum contained no significant population of antibodies which discriminated between these two variants within the K1 type (Fig. 7A).

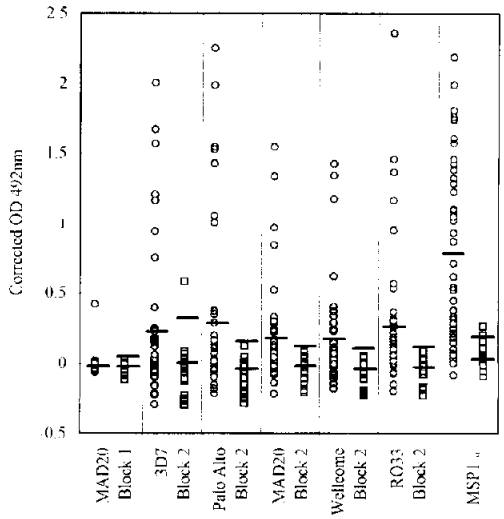


Fig. 5. Specific reactivity of recombinant MSP-1 antigens with serum IgG antibodies from malaria patients. Circles indicate sera from 43 Gambian patients and squares indicate sera from 37 non-exposed European controls; all sera were tested at 1:500 dilution in ELISA. Each point indicates reactivity of antibodies from a single individual, shown as OD units measured at 492 nm, corrected for background binding to GST control antigen alone. Horizontal bars indicate mean values for Gambian sera, or mean values and mean plus 2 standard deviations for European sera.

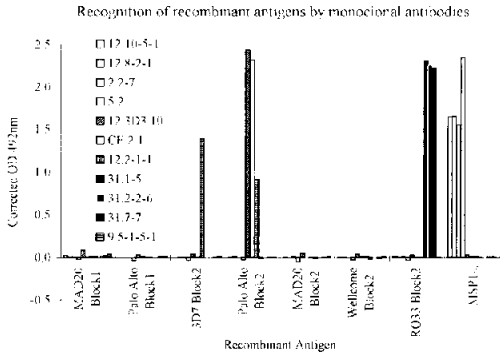


Fig. 4. Specific recognition of recombinant MSP-1 antigens by monoclonal antibodies against various regions of *P. falciparum* MSP-1. Each recombinant antigen was tested by ELISA for reactivity with the following antibodies: 12.3D3.10 and CE2.1 specific for tandem repeats of [SGT]n in Block 2 of Palo Alto [5, 24]; 12.2-1-1 to GASAQS sequences in Block 2 of K1 type [12,24]; 31.1, 31.2 and 31.7 to RO33 type of Block 2 [unpublished]; 2.2-7, 12.8-2, 12.10-5, and 5.2 to disulphide-dependent epitopes in MSP-$1_{19}$ [3,13]; 9.5-1-5-1, 13.2-3, 89.1 and 127B2-11 [2, 5, 12, 38] were negative control mAbs to epitopes in Blocks 3–7 within the MSP-183 fragment; only reactivity of the first of these control mAbs is shown.

In serum F8 (MAD20-type recognition), inhibition of antibody binding by both MAD20 and Wellcome competing variants, versus either as capture antigen, indicated that this serum contained antibodies directed against epitopes shared between the Wellcome and MAD20 Block 2 region (Fig. 7B). This serum also contained a residual population of antibodies which were specific for only the MAD20 Block 2 as shown by the incomplete inhibition of binding by the Wellcome variant as competitor antigen when the MAD20 variant is used as capture antigen.

Irrelevant competitor antigens of other types were used as controls in each case, and these did not inhibit the type-specific antibody-antigen interactions analysed above.

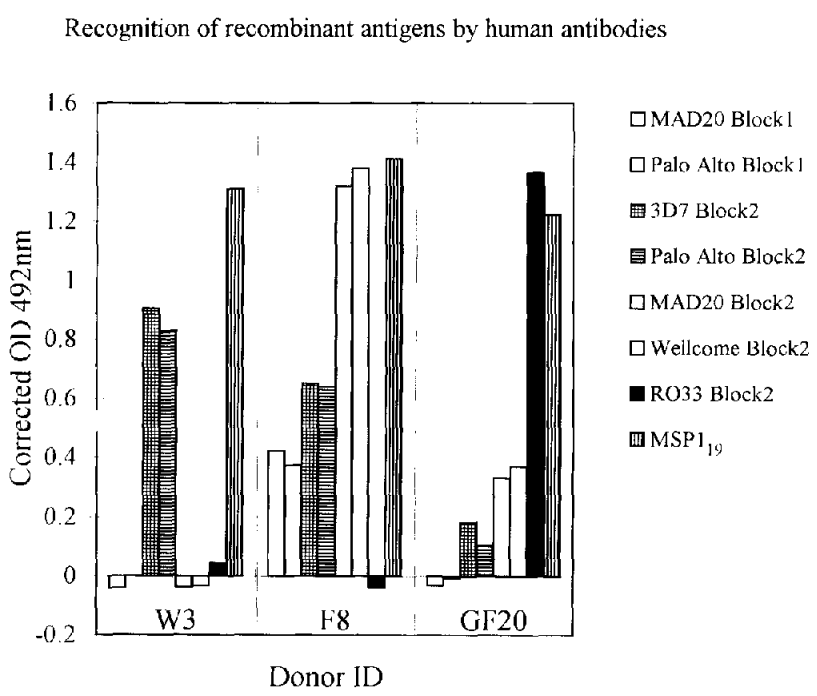


Fig. 6. Human IgG antibodies to Block 2 are type-specific. Antigens are denoted in the figure legend and identities of three serum donors chosen as typical examples are marked below each series of assays.

## 4. Discussion

Recombinant antigens representing the relatively conserved Block 1 region and five variants of the polymorphic Block 2 region of *P. falciparum* MSP-1 have been made that contain antigenic determinants similar to those of the native MSP-1 of the parasite. All the recombinant antigens contain amino acid sequences identical to the published sequences of the respective parasite variants of MSP-1 [7,9,30,31] with the exception of 3D7 [40], where minor differences were noted and corrected.

Monoclonal antibodies to MSP-1 reacted with the recombinant antigens as expected from their known specificities for the natural parasite products of various MSP-1 alleles (Fig. 4). The highly specific recognition of the appropriate recombinant antigens by each of the mAbs raised against MSP-1 of the parasite indicates that the recombinant antigens are in a form which mimics epitopes of the natural MSP-1 from parasites. The repetitive sequence of recombinant Palo Alto Block 2 is similar enough to contain two natural repetitive epitopes of the parasite protein.

Antibodies raised in mice against the relatively conserved Block 1 antigens recognised all tested variants of parasite-expressed MSP-1. In contrast, polyclonal murine antibodies against the recombinant Block 2 antigens showed strict specificity for only one of the three main types and were thus, able to discriminate between types effectively. However, these same antibodies were not able to discriminate between variants of the same type, despite significant differences between the internal repetitive sequences of these antigens. Antibodies raised in mice against recombinant Block 2 are thus directed primarily at shared epitopes within a type rather than against the polymorphic repeats found in the MAD20- and K1-like variants.

Recognition of the recombinant Block 2 antigens by serum IgG antibodies from malaria-exposed individuals from Africa appears similar to their recognition by the murine immune system,

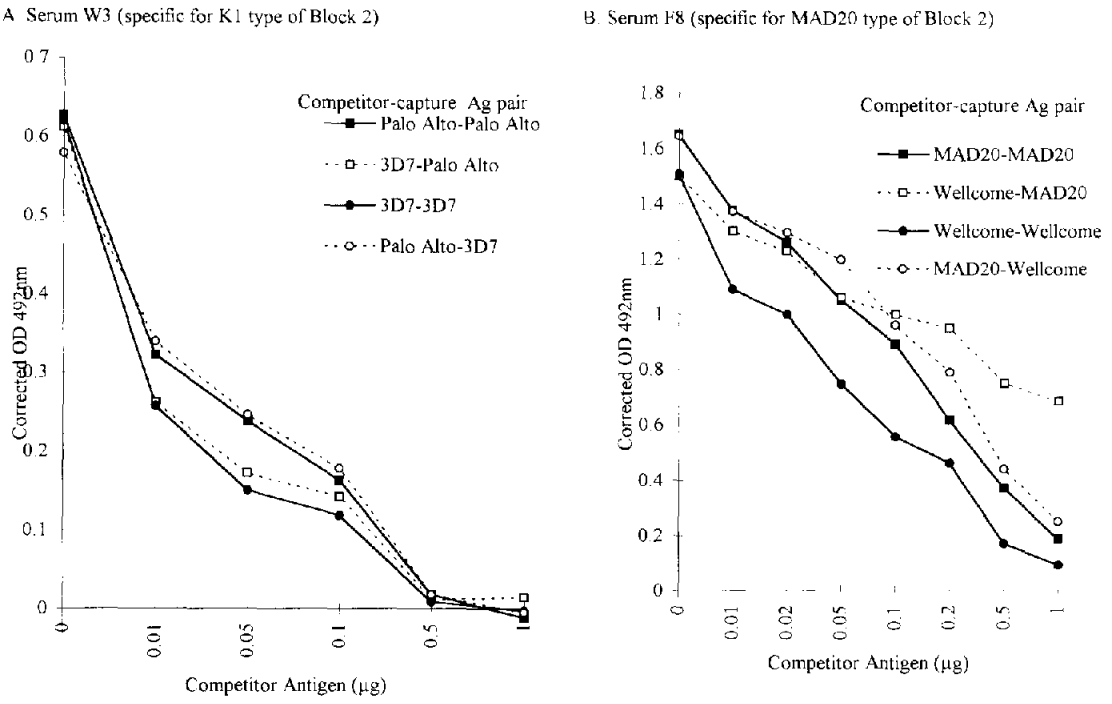


Fig. 7. Analysis of type-specific human antibodies against Block 2 of MSP-1 by competition ELISA. A, Serum from donor W3 (K1-type recognition) and B, Serum from donor F8 (MAD20-type recognition) used at 1:500 dilution. Legend in each panel indicates the pairs of competing antigens used, with the soluble competing antigen listed first and the well-bound capture antigen second. The capture antigen was coated at 50 ng/well. The increasing amount of competing antigen added to the diluted sera is indicated along the *X*-axis.

in that detectable human antibodies are type-specific but are primarily directed against shared epitopes within a type, rather than against polymorphic repetitive epitopes. All anti-Block 2 positive human sera identified so far (Fig. 5) show equivalent levels of reactivity with Block 2 antigens within any one type, and fail to discriminate well between antigens with different internal repetitive sequences. This result was extended here for two representative sera by competition ELISA to show that most of the human IgG reactive with one variant of the K1 (or MAD20) type can be inhibited from binding to that variant by a different variant of the same type. It therefore seems that in malaria-exposed individuals, the IgG responses to the Block 2 of the K1 or of the MAD20 type variants are directed predominantly against the flanking sequences that define the types rather than against the internal polymorphic repeats. Alternatively, unlike all existing mAbs, some human antibodies may recognise similar epitopes generated by non-identical but related repetitive sequences. The apparent scarcity of human antibodies specific for the repetitive parts of Block 2 is perhaps surprising, since such repetitive sequences have been proposed to contain 'immunodominant' B cell epitopes in other *P. falciparum* proteins [42]. It is well established that repeated structures within antigens crosslink surface immunoglobulins on B cells to provide activation signals independent of T cell help [43,44]. Repetitive structures of malaria proteins have been suggested to induce 'ineffectual' B cell responses which, in addition, might suppress the formation of antibodies to other more important epitopes on the same or other antigens [45]. In

contrast, it has been hypothesised that in the case of MSP-1, the extensive allelic diversity of repetitive sequences would make them less likely to be immunodominant [46]. Our results, the first experimental evidence on the matter, appear to support the latter hypothesis. However, the Block 2 antigens chosen for expression and study here represent only a fraction of the known sequence polymorphism in this region of the MSP-1 molecule and thus, it is not excluded that human sera do contain antibodies to variants of Block 2 repeats other than those included in our limited panel of recombinant antigens. In agreement with our results, Tolle et al. [28] showed in a survey of malaria exposed individuals in Mali that the Block 2 region of MSP-1 is immunogenic, but not immunodominant, since antibodies to other epitopes within the protein were found with equivalent frequency. Since their assay contained only one variant of each of the K1-like, MAD20-like, or RO33 types, they were unable to comment on the immunogenicity of variant-specific repeats per se.

In our experience, the frequencies at which IgG antibodies specific for the conserved Block 1 (0.02%) or the polymorphic Block 2 types (19–42%) of MSP-1 are detected in sera from malaria-exposed individuals are much lower than that of antibodies to the conserved C-terminal antigen $MSP\text{-}1_{19}$ (75%). For Block 2, this could be explained by a lower frequency with which any of the three types of Block 2 is likely to be encountered in naturally transmitted infections. In any series of *P. falciparum* infections of one individual, the same C-terminal fragment would be encountered every time, whereas the chance of infections with parasites expressing the same type, let alone the same variant, of the polymorphic Block 2 is considerably less. Thus, the frequency of exposure to a particular epitope (or set of epitopes) would be the main determinant of the frequency with which antibodies to that region are detected in naturally exposed individuals. Why the conserved Block 1 region rarely if ever provokes human antibody responses is more puzzling. Since conserved sequences in both our recombinant Block 1 antigens are sufficiently immunogenic to induce very reliably MSP-1-specific antibodies in mice, and are recognised by serum IgG of a few malaria-exposed individuals, the recombinant proteins do contain some conserved epitopes. Thus, it appears that such epitopes of Block 1 are not well recognised in the course of a natural infection and do not promote strong (or long lasting) IgG antibody responses. Whether human antibody response to Block 1 is limited to the production of IgM is currently being investigated.

These recombinant antigens are now being used in assessing the frequency, specificity and kinetics of the humoral immune response to both conserved and polymorphic N-terminal regions of MSP-1 and will enable analysis of the so-called 'strain'-specific immune responses induced during naturally transmitted malaria infections.

## Acknowledgements

We thank Dr Anthony Holder for the kind gift of the $MSP\text{-}1_{19}$ plasmid construct, Drs. J.A. Lyon, C.P. Locher and S.P Chang for monoclonal antibodies, and Kay Samuel for her invaluable help with the immunisation work. This work was supported by the Wellcome Trust.

## References

[1] Howard, R.J. and Pasloske, B.L. (1993) Target antigens for asexual malaria vaccine development. Parasitol. Today 9, 369–374.

[2] 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.

[3] 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.

[4] 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 surface antigens of *Plasmodium falciparum*. Parasitology 94, 199–208.

[5] Lyon, J.A., Geller, R.H., Haynes, D., Chulay, J.D. and Weir, 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.

[6] 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-inhibiting antibodies. J. Exp. Med. 172, 379–382.

[7] Holder, A.A., Lockyer, M.J., Odink, K.G., Sandhu, J.S., Riveros-Moreno, V., Nicholls, S.C., Hillman, Y., Davey, L.S., Tizard, M.L.V., Schwarz, R.T. and Freeman, R.R. (1985) Primary structure of the precursor to the three major surface antigens of *Plasmodium falciparum* merozoites. Nature 317, 270–273.

[8] Blackman, M.J. and Holder, A.A. (1992) Secondary processing of the *Plasmodium falciparum* merozoite surface protein-1 (MSP-1) by a calcium-dependent membrane-bound serine protease: shedding of MSP-$1_{33}$ as a noncovalently associated complex with other fragments of MSP-1. Mol. Biochem. Parasitol. 50, 307–316.

[9] Tanabe, K., MacKay, M., Goman, M. and Scaife, J.G. (1987) Allelic dimorphism in a surface antigen of the malaria parasite *Plasmodium falciparum*. J. Mol. Biol. 195, 273–287.

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

[11] Conway, D.J., Greenwood, B.M. and McBride, J.S. (1992) Longitudinal study of *Plasmodium falciparum* polymorphic antigens in a malaria-endemic population. Infect. Immun. 60, 1122–1127.

[12] 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.

[13] Siddiqui, W.A., Tam, L.Q., Kramer, K.J., Hui, G.S., Yamaga, K.M., Chang, S.P. Chan, E.B. 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.

[14] Hall, R., Hyde, J.E., Goman, M., Simmons, D.L., Hope, I.A., Mackay, M., Merkli, B., Richle, R. and Stocker, J. (1984) Major surface antigen of a human malaria parasite cloned and expressed in bacteria. Nature 311, 379–385

[15] Etlinger, H.M., Caspers, P., Matile, H., Schoenfeld, H.-J., Stueber, D. and Takacs, B. (1991) Ability of recombinant or native proteins to protect monkeys against heterologous challenge with *Plasmodium falciparum*. Infect. Immun. 59, 3498–3503.

[16] Holder, A.A., Freeman, R.R. and Nicholls, S.C. (1988) Immunisation against *Plasmodium falciparum* with recombinant polypeptides produced in *Escherichia coli*. Parasite Immunol. 10, 607–617.

[17] Herrera, M.A., Rosero, F., Herrera, S., Caspers, P., Rotmann, D., Sinigaglia, F. and Certa, U. (1992) Protection against malaria in Aotus monkeys immunised with a recombinant blood stage antigen fused to a universal T-cell epitope: Correlation of serum gamma interferon levels with protection. Infect. Immun. 60, 154–158.

[18] 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.

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

[20] Holder, A.A. and Riley, E.M. (1996) Human immune response to MSP-1. Parasitol. Today 12, 173–174.

[21] Patarroyo, M.E., Amador, R., Clavijo, P., Moreno, A., Guzman, F., Romero, P., Tascon, R., Franco, A., Murillo, L.A., Ponton, G. and Trujillo, G. (1988) A synthetic vaccine protects humans against challenge with asexual blood stages of *Plasmodium falciparum* malaria. Nature 332, 158–161.

[22] Amador, R., Moreno, A., Valero, V., Murillo, L., Mora, A., Rojas, M., Rocha, C., Salcedo, M., Guzman, F., Espejo, F., Nunez, F. and Patarroyo, M. (1988) The first field trials of the chemically synthesised malaria vaccine SPf66: safety, immunogenicity and protectivity. Vaccine 10, 179–184.

[23] Alessandro, U.D., Leach, A., Drakeley, C.J., Bennett, S., Olaleye, B.O., Fegan, G.W., Jawara, M., Langerock, P., George, M.O., Targett, G.A.T. and Greenwood, B.M. (1995) Efficacy trial of malaria vaccine SPf66 in Gambian children. Lancet 346, 462–467.

[24] Locher, C.P., Tam, L.Q., Chang, S.P., McBride, J.S. and Siddiqui, W.A. (1996) *Plasmodium falciparum*: gp195 tripeptide repeat-specific monoclonal antibody inhibits parasite growth in vitro. Exp. Parasitol. 84, 74–83.

[25] Müller, H-M., Früh, K., von Brunn, A., Esposito, F., Lombardi, S., Crisanti, A. and Bujard, H. (1989) Development of the human immune response against the major surface protein (gp190) of *Plasmodium falciparum*. Infect. Immun. 57, 3765–3769.

[26] Früh,K., Doumbo, O., Müller, H-M., Koita, O., McBride, J., Crisanti, A., Touré, Y. and Bujard, H. (1991) Human antibody response to the major merozoite surface antigen of *Plasmodium falciparum* is strain specific and short lived. Infect. Immun. 59, 1319–1324.

[27] Chizzolini, C., Dupont, A., Akue, J.P., Kaufman, M. H., Verdini, A.S., Pessi, A. and Del Giudice, G. (1988) Natural antibodies against three distinct and defined antigens of *Plasmodium falciparum* in residents of a mesoendemic area in Gabon. Am. J. Trop. Med. Hyg. 39, 150–156.

[28] Tolle, R., Früh, K., Doumbo, O., Koita, O., N'Diaye, M., Fischer, A., Dietz, K. and Bujard, H. (1993) A prospective study of the association between human hu-

moral immune response to Plasmodium falciparum blood stage antigen gp190 and control of malaria infections. Infect. Immun. 61, 40–47.

[29] Walliker, D., Quakyi, I.A., Wellems, T.E., McCutchan, T.F., Szarfman, A., London, W.T., Corcoran, L.M., Burkot, T.R. and Carter, R. (1987) Genetic analysis of the human malaria parasite Plasmodium falciparum. Science 236, 1661–1666.

[30] Chang, S.P., Kramer, K.J., Yamaga, K.M., Kato, A., Case, S.E. and Siddiqui, W.A. (1988) *Plasmodium falciparum*: gene structure and hydropathy profile of the major merozoite surface antigen (gp195) of the Uganda-Palo Alto isolate. Exp. Parasitol. 67, 1–11.

[31] Certa, U., Rotmann, D., Matile, H. and Reber-Liske, R. (1987) A naturally occurring gene encoding the major surface antigen precursor P190 of *Plasmodium falciparum* lacks tripeptide repeats. EMBO J. 6, 4137–4142.

[32] Trager, W. and Jensen, J.B. (1976) Human malaria parasites in continuous culture. Science 193, 673 675.

[33] Haynes, J.D., Diggs, C.L., Hines, F.A. and Desjardins, R.E. (1976) Culture of the human malaria parasite *Plasmodium falciparum*. Nature 263, 767–769.

[34] Fenton, B., Clark, J.T., Khan, C.M.A., Robinson, V.J., Walliker, D., Ridley, R., Scaife, J.G. and McBride, J.S. (1991) Structure and antigenic polymorphism of the 35- to 48- kilodalton merozoite surface antigen (MSA-2) of the malaria parasite *Plasmodium falciparum*. Mol. Cell. Biol. 11, 963–971.

[35] Smith, D.B. and Johnson, K.S. (1988). Single step purification of polypeptides expressed in *E. coli* as fusions with glutathione *S*-transferase. Gene 67, 31–40.

[36] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A laboratory manual. 2nd edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

[37] Burghaus, P.A. and Holder, A.A. (1994) Expression of the 19kDa carboxy-terminal fragment of *Plasmodium falciparum* merozoite surface protein-1 in *Escherichia coli* as a correctly folded protein. Mol. Biochem. Parasitol. 64, 165 169.

[38] Conway, D.J. and McBride, J.S. (1991) Population genetics of *Plasmodium falciparum* within a malaria hyperendemic area. Parasitology 103, 7–16.

[39] Hawkes, R. (1982) Identification of concavalin A-binding proteins after sodium dodecyl suphate gel electrophoresis and protein blotting. Anal. Biochem. 123, 143 146.

[40] Ranford-Cartwright, L.C. Balfe, P. Carter, R. and Walliker, D. (1991) Direct sequencing of enzymatically-amplified DNA of alleles of the merozoite protein MSA-1 gene from the malaria parasite *Plasmodium falciparum*. Mol. Biochem. Parasitol. 46, 185–188.

[41] Peterson, M.G., Coppel, R.G., McIntyre, P., Langford, C.J., Woodrow, G., Borwn, G.V., Anders, R.F. and Kemp, D.J. (1988) Variation in the precursor to the major merozoite surface antigens of *Plasmodium falciparum*. Mol. Biochem. Parasitol. 27, 291–302.

[42] Anders, R.F. (1986) Multiple cross-reactivities amongst antigens of *Plasmodium falciparum* impair the development of protective immunity against malaria. Parasite Immunol. 8, 529 539.

[43] Fuchs, S., Mozes, E., Maoz, A. and Sela, M. (1974) Thymus independence of a collagen-like synthetic polypeptide and of collagen and need for thymus and bone marrow cell cooperation in immune response to gelatin. J. Exp. Med. 139, 148–158.

[44] Hillman, K., Shapira-Nahor, O., Blackburn, R., Hernandez, D. and Golding, H. (1991) A polymer containing a repeating peptide sequence can stimulate T-cell independent IgG antibody production in vivo. Cell Immunol. 134, 1–13.

[45] Kemp, D.J., Coppel, R.L. and Anders, R.F. (1987) Repetitive proteins and genes of malaria. Annu. Rev. Microbiol. 41, 181–208.

[46] Schofield, L. (1991) On the function of repetitive domains in protein antigens of *Plasmodium* and other eukaryotic parasites. Parasitol. Today 7, 99–105.

[47] Holder, A.A., Blackman, M.J., Burghaus, P.A., Chappel, J.A., Ling, I.T., McCallum-Deighton, N. and Shai, S. (1992) A malaria merozoite surface protein (MSP-1)-structure, processing and function. Mem. Inst. Oswaldo. Cruz. 87, 37–42.