Antibodies that Inhibit Malaria Merozoite Surface Protein-1 Processing and Erythrocyte Invasion Are Blocked by Naturally Acquired Human Antibodies

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Summary

Merozoite surface protein-1 (MSP-1) of the human malaria parasite Plasmodium falciparum undergoes at least two endoproteolytic cleavage events during merozoite maturation and release, and erythrocyte invasion. We have previously demonstrated that mAbs which inhibit erythrocyte invasion and are specific for epitopes within a membrane-proximal, COOH-terminal domain of M SP-1 (MSP-1<sub>19</sub>) prevent the critical secondary processing step which occurs on the surface of the extracellular merozoite at around the time of erythrocyte invasion. Certain other anti–M SP-1<sub>19</sub> mAbs, which themselves inhibit neither erythrocyte invasion nor MSP-1 secondary processing, block the processing-inhibitory activity of the first group of antibodies and are termed blocking antibodies. We have now directly quantitated antibody-mediated inhibition of M SP-1 secondary processing and invasion, and the effects on this of blocking antibodies. We show that blocking antibodies function by competing with the binding of processing-inhibitory antibodies to their epitopes on the merozoite. Polyclonal rabbit antibodies specific for certain M SP-1 sequences outside of MSP-1<sub>19</sub> also act as blocking antibodies. Most significantly, affinity-purified, naturally acquired human antibodies specific for epitopes within the NH₂-terminal 83-kD domain of M SP-1 very effectively block the processing-inhibitory activity of the anti-M SP-1<sub>19</sub> mAb 12.8. The presence of these blocking antibodies also completely abolates the inhibitory effect of mAb 12.8 on erythrocyte invasion by the parasite in vitro. Blocking antibodies therefore (a) are part of the human response to malarial infection; (b) can be induced by M SP-1 structures unrelated to the M SP-1<sub>19</sub> target of processing-inhibitory antibodies; and (c) have the potential to abolish protection mediated by anti–M SP-1<sub>19</sub> antibodies. Our results suggest that an effective M SP-1<sub>19</sub>-based falciparum malaria vaccine should aim to induce an antibody response that prevents M SP-1 processing on the merozoite surface.

The development of an effective malaria vaccine has become a major public health challenge. The protozoan organisms responsible for the disease, members of the genus Plasmodium, have a complicated life cycle, and in the human host the parasite exists in at least four morphologically and antigenically distinct forms. As a result, in individuals exposed to malarial infection, the immune response against the parasite is complex, and several stages of the life cycle are being explored as potential targets for vaccine-mediated immune intervention. Acute clinical malaria, which is often life-threatening in the case of infection with Plasmodium falciparum, is associated with replication of the asexual blood-stage parasite in circulating erythrocytes. Human passive immunization studies using antibodies isolated from donors clinically immune to falciparum malaria have indicated that antimalarial IgG can prevent this replication (1–4), and surface components of the infected erythrocyte and invasive merozoite stage of the parasite have therefore been studied intensively for their ability to induce protective immune responses. The merozoite expresses a number of surface proteins, one or more of which are thought to mediate the initial interaction between parasite and host erythrocyte (5, 6). Recent work in this laboratory has focused on the proteolytic processing of merozoite surface protein-1 (M SP-1).<sup>1</sup> Initially synthesized as a large (~200 kD) precursor during intracellular merozoite development, M SP-1 is present on the surface of the released merozoite in the form of a multicomponent protein complex derived via proteolytic pro-

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1 Abbreviations used in this paper: EGF, epidermal growth factor; HRP, horseradish peroxidase–conjugated; MSP-1, merozoite surface protein-1; TLCK, tosyl-L-lysyl chloromethyl ketone.
cessing (7, 8). At some point between merozoite release and completion of erythrocyte invasion, the membrane-bound component (M SP-19) of this surface complex is further cleaved at a single site to form two fragments, M SP-13, and M SP-19. This results in the majority of the complex being shed from the parasite surface, leaving only M SP-19, which represents the extreme COOH-terminal end of the M SP-1 precursor and is comprised of two epidermal growth factor (EGF)-like motifs, to be taken into the invaded cell on the parasite surface (9-12). Significantly, this so-called secondary processing of M SP-1 is conserved across the genus (13-15) and invariably goes to completion when a merozoite successfully invades a red blood cell, suggesting that it is a necessary step in the invasion pathway.

Studies in the rodent Plasmodium nubaudi and Plasmodium yoelii malaria models have shown that passive immunization with certain anti-M SP-19 mAbs, or immunization with recombinant M SP-19, can afford an astonishing degree of protection against a blood-stage challenge infection (16-20). Consistent with this, a number of reports have shown that polyclonal antibodies (21, 22) or mAbs (9, 23, 24) specific for epitopes within the P. falciparum M SP-19 domain can prevent erythrocyte invasion by merozoites in vitro. To investigate the mechanisms involved in this invasion inhibition, we recently studied a panel of anti-M SP-19 mAbs, and found that those antibodies which most effectively prevent invasion can, upon binding to M SP-1 on the surface of intact P. falciparum merozoites, completely prevent secondary processing of the molecule. Furthermore, of those mAbs which do not affect the processing, some can interfere with the processing-inhibitory activity of the first group of antibodies (25). This second group of antibodies was referred to as blocking antibodies.

In this study we extend this work to show that blocking antibodies act by competing with processing-inhibitory mAbs for binding to the merozoite surface. We show that polyclonal antibodies raised against M SP-1 sequences outside of M SP-19 can also have blocking properties similar to those of the anti-M SP-19 mAbs previously identified. Of most significance, human antibodies specific to the NH2-terminal domain of M SP-1, affinity-purified from sera of individuals naturally exposed to falciparum malaria, are potent blocking antibodies which can completely abolish the activity of invasion-inhibitory antibodies in vitro. Our observations reveal a mechanism by which the parasite can avoid the action of a class of protective antibodies, and have important implications for the optimal design, evaluation, and administration of M SP-1-based malaria vaccines.

Materials and Methods

Polyclonal and monoclonal antibodies. M urine anti-M SP-19 mAbs 2.2, 7.5, 12.8, 12.10, 111.4, 117.2, 1E1, 2F10, 7E5, 8A12, and 12D11; the anti-M SP-13 mAB 89.1, and the mAB 25.1, which is specific for P. yoelii M SP-1; and the human anti-M SP-13 mAB X 509 have all been previously described (7, 9, 10, 25-27). All mAbs were purified by affinity chromatography on protein A- or protein G-Sepharose (Pharmacia Biotech, St. Albans, Hertfordshire, UK) before use (28). A panel of polyclonal anti-M SP-1 anti-sera was raised in rabbits against defined regions of M SP-1 expressed as fusion proteins in Escherichia coli (8); IgG was purified from these sera by ion exchange chromatography on DEAE Sephadex (Pharmacia Biotech) using standard methods (28). The polyclonal rabbit antisera reactive with the M SP-13 fragment of the W elcome M SP-1 (R.b anti-M SP-13) was raised against a recombinant protein expressing a 93-amino acid region from within the N H2-terminal half of M SP-19; therefore, the antibodies recognize both M SP-19 and M SP-13, and show absolutely no reactivity with M SP-19 (13). Pooled human serum obtained from adult Gambian donors clinically immune to falciparum malaria was a kind gift of Dr. Hilton Whittle (Medical Research Council Laboratories, Fajara, The Gambia, West Africa). Human serum from European donors who had never been exposed to malaria (nonimmune sera) was obtained from the Blood Transfusion Centre (Colindale, U.K.) and pooled.

Preparation of recombinant antigens. Production of a recombinant pGEX-3X plasmid (29) to express the M SP-19 domain of the P. falciparum (Welcome strain) M SP-1 fused to Staphylococcus japonicum glutathione S-transferase has been described previously (26). Fusion protein was adsorbed to glutathione agarose (Sigma Chemical Co., St. Louis, MO), and the malarial portion (rM SP-19) cleaved in situ from the carrier protein (30) by overnight incubation with Factor Xa (Boehringer Mannheim, Mannheim, Germany) at 4°C. Eluted protein was further purified by gel filtration on PBS on Sephadex G50 Superfine (Pharmacia Biotech), and concentrated by ultrafiltration using a YM1 membrane (Amicon, Ltd., Stonehouse, Glocs., U.K.).

Recombinant expression plasmid pM E6 encodes Leu 20 to A sp2 of the P. falciparum W elcome strain M SP-1 gene (numbering according to reference 31), as an N H2-terminal fusion with β-galactosidase (8). Fusion protein (also referred to as pM E6; reference 8) was purified by affinity chromatography on p-amino-phenyl-p-d-thiogalactopyranoside-agarose (32) and stored as a precipitate in 50% (wt/vol) ammonium sulfate.

Radiolabeling of Antibodies. Protein G- purified mAbs 12.8, 12.10, and X 509, and purified rabbit anti-IgG antibodies (Seralab, Ltd., Sussex, U.K.) were labeled at 4°C with 35S-methionine and cysteine (Pro-MixTM; Amersham International, Little Chalfont, U.K.) and pooled.

When required, schizont-enriched cultures were metabolically labeled with 35S-methionine and cysteine (Pro-mixTM; Amersham International, Little Chalfont, U.K.), placed back into culture in medium containing 0.5% (wt/vol) AlbumaxTM (GIBCO})
BR L, Paisley, U K), and allowed to undergo merozoite release in the presence of fresh erythrocytes as previously described (10). Labeled M SP-133 was immunoprecipitated from harvested culture medium using mAb X 509 coupled to Sepharose, and analyzed by SDS-PAGE and fluorography as previously described (10, 11). When appropriate, ring-stage parasitemia in cultures after reinfection was assessed by microscopic examination of Giemsa-stained thin blood films.

Quantitation of Antibody-mediated Inhibition of M SP-1 Secondary Processing. Analysis and quantitation of secondary processing of M SP-1 in merozoite preparations was by modification of an assay described previously (13, 34). Washed merozoites were resuspended in ice-cold 50 mM Tris-HCl, pH 7.5, containing 10 mM CaCl2 and 2 mM MgCl2, supplemented with the following protease inhibitors: antipain, leupeptin, aprotinin, and TLCK (reaction buffer). Aliquots of ~10⁸ merozoites were dispensed into 1.4-ml Eppendorf tubes on ice, and the parasites were pelleted in a microfuge at 12,000 × g for 2 min at 4°C. The buffer was aspirated, and individual merozoite pellets were resuspended on ice in 20 μl of reaction buffer further supplemented with protease inhibitors or antibodies as appropriate. Merozoites were maintained on ice for 15 min to allow antibody binding, then transferred to a 37°C water bath for 1 h to allow processing to proceed. Assays always included the following controls: a "positive processing" control sample of merozoites, resuspended in reaction buffer only; a negative "no processing" sample of merozoites, resuspended in reaction buffer plus 1 mM PM SF; and a zero time (0 h) control, in which processing was immediately stopped before the 37°C incubation step by the addition of an equal volume of 2% (vol/vol) NP-40 (BDH Chemicals, Ltd., Poole, UK; reference 13).

Processing was stopped by the addition of 20 μl of 2% NP-40. Samples were vortexed and extracted on ice for 1 h, then centrifuged at 12,000 × g for 15 min, and then the supernatant was further diluted (usually 100-fold) in coating buffer. Six microliters of blocking antibodies would be readily apparent. Plates were incubated for a further 2 h at room temperature, then washed as previously described (35). In preliminary experiments, titration curves obtained using anti-M SP-19 mAbs in the two ELISA systems (m SP-19 and merozoite sonicate) were indistinguishable.

Competitive RIA. A competitive solid-phase RIA was used to determine whether or not anti-M SP-1 mAbs or rabbit antibodies could competitively block the binding of processing-inhibitory mAbs 12.8 and 12.10 to their epitopes. Wells of polyvinyl chloride microtiter plates (Falcon Labware, Becton Dickinson and Co., Oxnard, CA) were coated overnight at 4°C with 100 μl of merozoite antigen sonicate, or rM SP-19 at a final concentration of 10 μg ml⁻¹ in coating buffer. Plates were then washed three times in PBS/T and treated overnight at 4°C with PBS/T containing 1% (wt/vol) bovine serum albumin (PBS/T/BSA). The plates were then washed and 50 μl PBS/T/BSA containing serum or purified antibody at a saturating concentration (predetermined by ELISA; see above) was added to wells in triplicate. Plates were incubated for 2 h at room temperature, then washed again, and 50 μl of optimally diluted radiolabeled mAb 12.8 or 12.10 was added in PBS/T/BSA. Optimal concentrations of radiolabeled mAbs were determined in preliminary radioimmune titration assays; the final concentration of radiolabeled mAbs used in the competitive RIAs corresponded to those in the linear part of the dose-response curve, so that changes in 12.8 and 12.10 binding in the presence of blocking antibodies would be readily apparent. Plates were incubated for a further 2 h at room temperature, then washed as before. Individual wells were excised and counted for 1 min in a gamma counter. Samples were routinely assayed in triplicate, and the binding of radiolabeled mAbs was expressed as a percentage of that obtained in the absence of pretreatment of wells.

Affinity Purification of Human Antibodies Reactive with pM E6. Purified pM E6 protein was bound to cyanogen bromide-activated Sepharose 4B (Pharmacia Biotech) at 5 mg ml⁻¹, allowed to form 30 ml of pooled serum derived from adult Gambian donors was diluted 1:4 in 50 mM Tris-HCl, pH 8.0, containing 0.02% (wt/vol) sodium azide, clarified by passage through a 0.45-μm filter, then passed over a 5-ml affinity column at a flow rate of 10 ml h⁻¹. The column was washed extensively in 50 mM Tris-HCl, pH 8.0, and bound IgG was eluted in the same buffer containing 8 M urea. Samples of eluate fractions were subjected to SDS-PAGE under reducing conditions.
Results

Development and Validation of an Assay to Quantitate Antibody-mediated Inhibition of MSP-1 Processing. In previous work, a panel of MSP-119-specific mAbs was tested for their ability to interfere with secondary processing of MSP-1 (25) using a Western blot-based procedure that allowed only a semiquantitative estimate of processing inhibition. To improve the assay for this study, a radioiodinated, affinity-purified anti-rabbit IgG was used. Autoradiography of the probed blots allowed visualization of bands corresponding to MSP-142 and its processed product MSP-133, and the amount of antibody bound to each was determined by direct counting in a gamma counter. When an extract of incubated merozoites was analyzed by this method, the radioactivity associated with each of the MSP-133 and MSP-142 bands on the blot was, within limits imposed by the protein binding capacity of the blotting membrane, directly proportional to the volume of merozoite extract loaded on the gel (not shown). This linear relationship did not hold if an extract of more than \( \sim 2 \times 10^8 \) merozoites was loaded per track, and in all subsequent experiments this limit was not exceeded. During a 1-h incubation of merozoites, the observed decrease over time in the number of counts associated with MSP-142 (due to processing of the polypeptide) was concomitant with a corresponding increase in the number of counts associated with MSP-133, and at least 50% of the MSP-142 underwent processing in this period (data not shown). These results are in accordance with previous data showing stoichiometric conversion of MSP-142 to MSP-133 (13), and that accurate quantitation of MSP-1 processing is possible with this assay. In a typical assay, the number of cpm associated with the MSP-133 band in the zero time (0 h) control and the positive processing control sample (incubated for 1 h in reaction buffer only; see Materials and Methods) was 20 and 1,300 cpm, respectively (data not shown).

The assay was used to quantify MSP-1 secondary processing and its inhibition by a panel of anti-MSP-119 mAbs. Washed FCB-1 merozoites were incubated on ice in the presence of individual purified mAbs, then transferred to 37°C for 1 h to allow processing to occur. MSP-142 processing in the individual samples was then assessed using the above protocol. Fig. 1 shows that mAb 12.8, which recognizes a conserved epitope in the first EGF-like motif of MSP-119 (36, 37), inhibited processing by 96% of the control value, whereas mAb 12.10, which recognizes an epitope formed by the two EGF-like motifs together (37), inhibited processing by 98%. Monoclonal antibody 1E1 showed no processing-inhibitory activity in this assay system. Interestingly, our earlier data obtained using a semiquantitative Western blot-based assay indicated that mAb 1E1 appeared to induce abnormal processing rather than preventing the processing; in addition, mAb 1E1 does not prevent erythrocyte invasion in in vitro cultures of P. falciparum (25). Antibodies 8A12 and 117.2 inhibited MSP-142 processing by 18 and 12%, respectively, whereas mAbs 111.4, 12D11, and 7E5 did not detectably prevent processing. Neither mAb 89.1, which recognizes an epitope within the NH2-terminal domain of MSP-1 (MSP-183), nor the anti-P. yoelii MSP-1 mAb 25.1, had any effect on the processing (data not shown). These results confirm that mAbs 12.8 and 12.10 are potent inhibitors of MSP-142 processing. In similar assays using merozoites of the P. falciparum clone T9/96, which expresses the alternative dimorphic form of MSP-1 (31), but retains the nonpolymorphic epitopes recognized by mAbs 12.8 and 12.10 (38), both mAbs showed similarly potent processing inhibition activity (data not shown).

Blocking Antibodies Act by Competitively Preventing the Binding of Processing-inhibitory mAbs to Merozoites. Previous work (25) has indicated that a number of anti-MSP-119 mAbs, which themselves do not inhibit MSP-1 processing, can block the ability of mAbs 12.8 and 12.10 to interfere with the processing. Although the mechanism of this blocking activity was not elucidated, the most likely explanation is that a blocking antibody can compete with a processing-inhibitory antibody for binding to MSP-1 on the merozoite surface. In this study, this hypothesis was directly tested using a competitive RIA to investigate the effects of known blocking antibodies on binding of processing-inhibitory antibodies to native, merozoite-derived MSP-1. Wells of 96-well polyvinyl chloride plates coated with merozoite antigen extract were incubated with anti-MSP-119
mAbs at saturating concentrations. The plates were then washed and an optimal concentration of radiiodinated mAb 12.8 or 12.10 was added. After further incubation, plates were washed and individual wells were counted directly in a gamma counter. Fig. 2 shows that antibodies known to interfere with the processing activity of mAbs 12.8 and 12.10 to FCB-1 merozoite-derived MSP-1 are competitively prevented by certain other anti-MSP-1 mAbs. Plates coated with a merozoite antigen extract were preincubated in triplicate with either no antibody (noAb; control wells), or with predetermined saturating concentrations of mAbs 12.10, 12.8, 2.2, 111.4, 7.5, 1E1, or 89.1. The effects of this pretreatment on binding of radiiodinated mAbs 12.8 (A) or 12.10 (B) to the immobilized antigen was then assessed. All samples were tested in triplicate. Blocking activity of individual mAbs was calculated as described in Materials and Methods.

**Figure 2.** The binding of processing-inhibitory mAbs 12.8 and 12.10 to FCB-1 merozoite-derived MSP-1 is competitively prevented by certain other anti-MSP-1 mAbs. Plates coated with a merozoite antigen extract were preincubated in triplicate with either no antibody (noAb; control wells), or with predetermined saturating concentrations of mAbs 12.8, 12.10, 2.2, 111.4, 7.5, 1E1, or 89.1. The effects of this pretreatment on binding of radiiodinated mAbs 12.8 (A) or 12.10 (B) to the immobilized antigen was then assessed. All samples were tested in triplicate. Blocking activity of individual mAbs was calculated as described in Materials and Methods.

The above results showed that the binding of processing-inhibitory antibodies to MSP-1 can be specifically prevented by the interaction of other antibodies with the same polypeptide, and explained how blocking antibodies interfere with the processing-inhibitory activity of mAbs 12.8 and 12.10. Interestingly, Wilson et al. (38) found that mAb 13.2, which recognizes an epitope within the NH₂-terminal domain of MSP-1, prevents the binding of mAb 12.8 to intact MSP-1, raising the possibility that antibodies specific to other components of the MSP-1–derived, merozoite surface protein complex might have blocking activity. To investigate this possibility, a series of rabbit antibodies, raised against recombinant proteins corresponding to regions covering all of MSP-1 (reference 8; Fig. 3) were tested for their ability to competitively prevent recognition of merozoite-derived MSP-1 by mAbs 12.8 and 12.10. Fig. 4 shows that binding of radiiodinated mAbs 12.8 and 12.10 to the merozoite antigen was significantly blocked by some but not all of the polyclonal antibodies. The fact that rabbit antibodies raised against pM E12, 16, and 20 were able effectively to block binding was not unexpected, due to the presence of the 12.8 and 12.10 epitopes within the sequence of the recombinant proteins used to raise these rabbit sera. However, it was found that antibodies raised against constructs corresponding to domains of MSP-1 outside the COOH-terminal region also showed potent blocking activity; in particular, the anti-pM E6, anti-pM E14, and anti-pM E3 sera inhibited binding of mAb 12.8 to the immobilized antigen by 68, 48, and 91%, respectively, and the rabbit anti-pM E14, anti-pM E1, and anti-pM E3, but not the anti-pM E6 antibodies, significantly prevented binding of mAb 12.10. These results show that polyclonal antibodies specific for fragments of the MSP-1 complex other than MSP-119 can act as blocking antibodies.
Naturally acquired human antibodies specific for epitopes within the NH2-terminal domain of MSP-1 block the activity of processing-inhibitory antibodies. Antibodies which prevent MSP-1 processing and erythrocyte invasion may be involved in mediating protection against blood-stage parasitemia. If antibodies induced to other domains of MSP-1 can block the activity of processing-inhibitory antibodies specific for MSP-119, their presence in human sera may be disadvantageous to the host. In light of the above data, it was decided to investigate the ability of naturally acquired antibodies specific for the region of MSP-1 corresponding to pME6, to block the processing-inhibitory activity of mAbs 12.8 and 12.10. This particular construct was chosen because pME6 is readily soluble (8), and the E. coli clone which expresses pME6 does so at very high levels. Human antibodies reactive with pME6 were isolated from pooled Gambian adult immune serum by affinity chromatography on immobilized pME6 fusion protein. The eluted Ig was judged to be >98% pure as assessed by SDS-PAGE under reducing conditions (data not shown). The Ig was concentrated by ultrafiltration and assayed by immunoblot for reactivity with FC-B-1 merozoite polypeptides. Strong reactivity was observed with only two merozoite polypeptides of ~83 and 195 kD (Fig. 5); these most likely correspond to MSP-183 and the residual MSP-1 precursor protein. Note that the purified antibodies showed no reactivity with the MSP-142 and MSP-119 species (Fig. 5, arrows). In confirmation of this, analysis of the affinity-purified Ig by indirect immunofluorescence showed strong reactivity with FC-B-1 merozoite polypeptides which contain only MSP-183 and the residual MSP-1 precursor protein. Note that since the pME6 construct covers much of the highly conserved MSP-1 block 3 domain, as well as all of the conserved block 5 (see Fig. 3), antibodies against pME6 would be expected to recognize both allelic forms of MSP-1.

The ability of the affinity-purified human antibodies to block the processing-inhibitory effects of mAbs 12.8 and 12.10 was then assessed. Merozoites were incubated on ice in the presence or absence of the human anti-pME6 antibodies, and control wells were pretreated either with mAbs 12.8 or 12.10, or with buffer alone (No Ab) or with a nonimmune rabbit serum (NI Rs) at a final dilution of 1:100. All samples were assayed in triplicate, and SE bars are indicated.
and SE bars are shown.

nated mAb 12.8 or 12.10 to bind. All samples were assayed in triplicate, lent final antibody concentration, before assessing the ability of radioiodi-

out the additional presence of affinity-purified anti-pME6 antibodies. (Figure 6. Affinity-purified, naturally acquired human anti-pME6 anti-

bodies are potent blocking antibodies. (A) Equal aliquots of washed FCB-1 merozoites were solubilized directly into detergent (0 h control), or pre-

incubated either with reaction buffer only or with affinity-purified human anti-pME6 antibodies at a final concentration of 300 µg ml⁻¹. An equal concentration of mAb 12.10 or 12.8 was then added to some samples as shown, and processing was allowed to proceed for 1 h in all but the 0 h control. Inhibition of M SP-1 processing mediated by mAb 12.8 alone (98%) was almost completely reversed by preincubation with the anti-pME6 an-

tibodies, whereas the inhibition of processing mediated by mAb 12.10 alone (97%) was completely unaffected by preincubation with anti-pME6 antibodies. (B) RIA plates coated with merozoite antigen were pretreated with nonradioactive mAb 12.10 or 12.8 at a saturating concentration (100 µg ml⁻¹), or affinity-purified anti-pME6 antibodies at a saturating concentra-

tion (300 µg ml⁻¹), or nonimmune human serum (NI Hs) at an equiva-

lent final antibody concentration, before assessing the ability of radiodini-

ated mAb 12.8 or 12.10 to bind. All samples were assayed in triplicate, and SE bars are shown.

These data clearly show that the binding of antibodies specific to one component of the M SP-1-derived merozoite surface complex can interfere with the binding of anti-

bodies to another component of the complex. Erythrocyte invasion by the malaria merozoite is rapid, going to com-

pletion within seconds of the initial interaction between parasite and red cell surface (39). Over such a short time span, could the presence of blocking antibodies interfere with the ability of processing-inhibitory antibodies to bind to the merozoite surface and prevent both processing and invasion? To address this question directly in an in vitro system, a series of invasion experiments was performed. Mature, biosynthetically radiolabeled T 9/96 schizonts were washed and placed in culture with fresh red cells. Merozoite release and red cell invasion were then allowed to proceed in the presence or absence of mAbs 12.8 and 12.10, with or without the additional presence of affinity-purified anti-pME6 human antibodies. The overall efficiency of invasion was as-

sessed by counting the number of new ring stage parasites formed over the course of the experiment; M SP-1 processing in individual samples was subsequently assessed by direct immunoprecipitation of M SP-1₁₉ from the culture supernatants using mAb X 509 coupled to Sepharose. In preliminary dose-response experiments, a concentration of ≈400 µg ml⁻¹ of either mAb 12.10 (Fig. 7 A) or mAb 12.8 (data not shown) was sufficient to reduce the amount of M SP-1₁₉ release to a level of inhibition seen in the presence of 5 mM EGTA, a potent inhibitor of M SP-1 secondary processing (11). The results of a typical experiment (of a total of three independent experiments) investigating the effects of the anti-pME6 blocking antibodies on the activity of mAbs 12.8 and 12.10 are presented in Fig. 7 B. In isolation, mAbs 12.8 and 12.10 virtually abolished both invasion (Fig. 7 B, bottom) and M SP-1₁₉ release (Fig. 7 B, top). However, in the presence of equal concentrations of the anti-pME6 hu-

man antibodies, the effects of mAb 12.8, but not of 12.10, were completely reversed (Fig. 7 B, lanes 5 and 7). Neither the anti-pME6 antibodies alone nor mAb 89.1 alone had any effect on either processing or invasion (Fig. 7 B, lanes 3 and 8), and mAb 89.1 exhibited no blocking activity (Fig. 7 B, lanes 9 and 10). These results unambiguously demonstrate that, under conditions of active release of viable merozoites, mAbs 12.8 and 12.10 effectively prevent both M SP-1 processing and erythrocyte invasion, and this activity can be efficiently abrogated by the presence of human blocking antibodies.

Discussion

Four major conclusions can be drawn from this study. First, blocking antibodies function by competitively pre-

venting the binding of processing-inhibitory antibodies to the merozoite surface, and can be effective under condi-

tions of active merozoite release and erythrocyte invasion. Second, blocking activity can be mediated not only by anti-

bodies specific for the M SP-1₁₉ domain, but also by antibod-

ies binding to polypeptides other than the M SP-1₁₉ tar-

get of processing-inhibitory antibodies; here we have shown that antibodies reactive with a region within M SP-1₉₃, a polypeptide derived from the NH₂-terminal domain of the M SP-1 precursor, possess potent blocking activity. Antibodies against other fragments of the merozoite surface complex, possibly including the non-M SP-1-derived components of it (12, 40), may also mediate blocking activity; indeed, our present data suggest that antibodies against the region of M SP-1 represented by pME14 possess significant blocking activity (Fig. 4). Third, human blocking antibod-

ies can be induced by natural exposure to malarial infec-

tion. Fourth, if prevention of M SP-1 processing is a major mechanism by which anti-M SP-1₁₉ antibodies exert their effect on erythrocyte invasion by the P. falciparum merozo-

ite, then the protective potential of inducing such antibod-

ies by vaccination could be impaired by a preexisting or si-

multaneously induced blocking antibody response directed against M SP-1₁₉ itself, or other components of the M SP-1 protein complex.
MSP-1 is receiving increasing interest as a candidate antigen for a blood-stage malaria vaccine. Experimental passive immunization and direct immunization-challenge studies focusing on the protective capacity of anti–MSP-119 antibody responses have been substantiated by epidemiological studies in malaria-endemic areas showing a significant positive association between levels of serum antibodies against MSP-1 and resistance to morbidity associated with falciparum malaria (41, 42). However, the seroepidemiological data are ambiguous. For example, there is not a simple relationship between seropositivity and clinical immunity, and there is extensive evidence that parasite replication can take place in vivo in the presence of substantial levels of circulating anti-MSP-1 antibody (41-43). With no clear consensus on either the mechanism(s) by which anti-MSP-1 antibodies control replication of the parasite or the biological function of MSP-1 on the merozoite surface (6, 44), the effector mechanisms required of an optimally protective anti-MSP-1 immune response have been unclear. Given the imminent availability of first generation MSP-119-based vaccines for clinical evaluation, there is a pressing need to define indicators of a protective anti-MSP-1 response which are amenable to quantitative serological assay (45).

We propose that antibodies specific for the P. falciparum MSP-119 domain prevent merozoites from invading erythrocytes primarily by interfering with MSP-1 secondary processing. This hypothesis is supported by the apparently absolute correlation between antibody-mediated processing-inhibitory activity and invasion inhibitory activity; of a total of 11 distinct anti–M. falciparum MSP-119 mAbs tested to date, only mAbs 12.8 and 12.10 exhibit either activity (this study, reference 25, and our unpublished data). The hypothesis would explain the observed absence of a straightforward correlation between total serum anti–MSP-119 antibody levels and immunity to blood-stage parasitemia in individuals naturally exposed to malaria, since many anti-MSP-119 antibody specificities clearly have no effect on MSP-119, further complicates attempts to predict the protective capacity of an antibody response to MSP-119 in the presence of a polyclonal response against the total MSP-1. Therefore, the validity of the continued use of simple ELISA-based assays in epidemiological studies may be questionable. We tentatively conclude that only a functional assay, such as one measuring MSP-1 processing inhibition, or the effect on invasion of affinity-purified antibodies (43), can provide an assessment of the overall protective capacity of anti–MSP-119 mAbs can prevent M SP-1 and erythrocyte invasion in in vitro culture, and can be rendered ineffective by the simultaneous presence of anti-pME6 blocking antibodies. (A) Dose-response effect of mAb 12.10 on M SP-1 secondary processing. Metabolically radiolabeled T9/96 schizonts were supplemented with fresh erythrocytes and medium to obtain a parasitemia of ~2% and a hemotocrit of 1%. The culture was then divided into equal aliquots and incubated at 37°C in the presence of 5 mM EGTA as control inhibitor (lane 1), or mAb 12.10 at a final concentration of 1 μg/ml-1 (lane 2), 2 μg/ml-1 (lane 3), 500 μg/ml-1 (lane 4), 400 μg/ml-1 (lane 5), 300 μg ml-1 (lane 6), 200 μg ml-1 (lane 7), 100 μg ml-1 (lane 8), or no antibody (lane 9). Schizont rupture and merozoite release were then allowed to proceed for 6 h, and culture supernatants were analyzed by immunoprecipitation using mAb X509 coupled to Sepharose for the presence of MSP-1 (8) Blocking anti-pME6 antibodies reverse the processing-inhibitory (top) and invasion-inhibitory (bottom) activity of mAb 12.8. Cultures containing metabolically radiolabeled T9/96 schizonts prepared as described above were incubated in the presence of 5 mM EGTA (lane 1), 10% (vol/vol) nonimmune human serum (lane 2), anti-pME6 antibodies (lane 3), mAb 12.8 (lane 4), mAb 12.8 plus anti-pME6 antibodies (lane 5), mAb 12.10 (lane 6), mAb 12.10 plus anti-pME6 antibodies (lane 7), mAb 89.1 (lane 8), mAb 89.1 plus mAb 12.8 (lane 9) and mAb 89.1 plus mAb 12.10 (lane 10). In this case all antibodies were added to a final concentration of 400 μg ml-1. Analysis of 6-h culture supernatants by immunoprecipitation with mAb X509 (8, top) was as above, and in addition erythrocyte invasion in individual cultures was assessed by counting the number of ring-stage parasites in 5,000 red cells, in triplicate (8, bottom). Invasion is expressed as a percentage of the ring-stage parasitemia (10%) obtained in a control culture with no additions (data not shown).

Figure 7. Processing-inhibitory anti–M SP19 mAbs can prevent M SP-1 and erythrocyte invasion in in vitro culture, and can be rendered ineffective by the simultaneous presence of anti-pME6 blocking antibodies. (A) Dose-response effect of mAb 12.10 on M SP-1 secondary processing. Metabolically radiolabeled T9/96 schizonts were supplemented with fresh erythrocytes and medium to obtain a parasitemia of ~2% and a hemotocrit of 1%. The culture was then divided into equal aliquots and incubated at 37°C in the presence of 5 mM EGTA as control inhibitor (lane 1), or mAb 12.10 at a final concentration of 2 μg/ml-1 (lane 2), 1 μg/ml-1 (lane 3), 500 μg/ml-1 (lane 4), 400 μg/ml-1 (lane 5), 300 μg/ml-1 (lane 6), 200 μg/ml-1 (lane 7), 100 μg/ml-1 (lane 8), or no antibody (lane 9). Schizont rupture and merozoite release were then allowed to proceed for 6 h, and culture supernatants were analyzed by immunoprecipitation using mAb X509 coupled to Sepharose for the presence of MSP-1. (B) Blocking anti-pME6 antibodies reverse the processing-inhibitory (top) and invasion-inhibitory (bottom) activity of mAb 12.8. Cultures containing metabolically radiolabeled T9/96 schizonts prepared as described above were incubated in the presence of 5 mM EGTA (lane 1), 10% (vol/vol) nonimmune human serum (lane 2), anti-pME6 antibodies (lane 3), mAb 12.8 (lane 4), mAb 12.8 plus anti-pME6 antibodies (lane 5), mAb 12.10 (lane 6), mAb 12.10 plus anti-pME6 antibodies (lane 7), mAb 89.1 (lane 8), mAb 89.1 plus mAb 12.8 (lane 9) and mAb 89.1 plus mAb 12.10 (lane 10). In this case all antibodies were added to a final concentration of 400 μg ml-1. Analysis of 6-h culture supernatants by immunoprecipitation with mAb X509 (8, top) was as above, and in addition erythrocyte invasion in individual cultures was assessed by counting the number of ring-stage parasites in 5,000 red cells, in triplicate (8, bottom). Invasion is expressed as a percentage of the ring-stage parasitemia (10%) obtained in a control culture with no additions (data not shown).
an anti-MSP-1 antibody response. The critical test of our hypothesis will be the predictive power of the assay; opportunities to evaluate this will arise from immunization trials in naive primates or humans with MSP-119 or MSP-142–based vaccines in which significant protection is achieved (46, 47). This is a major priority, and work towards it is in progress. A further implication of our hypothesis is that, for an MSP-119–based vaccine to be effective, its design or mode of administration should be such that the overall balance of the induced antibody response is towards processing-inhibitory antibody specificities, rather than blocking specificities. Selectively inducing this type of functional antibody response may be the major challenge in MSP-119–based vaccine development.

How do antibodies specific for the NH2-terminal domain of MSP-1 (MSP-183) exert blocking activity? There are no published structural data on the merozoite surface complex. However, treatment of intact merozoites with the bifunctional, cleavable cross-linker 3,3'-dithiobis(sulfosuccinimidylpropionate) results in almost quantitative cross-linking of the MSP-183 and MSP-142 components of the complex (Blackman, M.J., unpublished data), suggesting that at least in the conformation adopted by the membrane-bound form of the complex, these two polypeptides are spatially close. Given the additional fact that the molecular mass of an IgG molecule is not much less than that of the monomeric M SP-1 complex, the observation of steric competition between anti-MSP-183 and anti-MSP-142 antibodies is perhaps unsurprising. However, it is not clear why polyclonal antibodies reactive with the part of MSP-183 represented by pM E6 should selectively block binding of mAb 12.8, but not 12.10; presumably the two processing-inhibitory mAbs adopt quite distinct orientations on binding. Whatever the case, this work has provided the first experimental evidence that antibodies against one part of a merozoite surface protein can "shield" the parasite from the potentially harmful effects of antibodies directed against another part of the same surface protein. MSP-183 is known to be immunogenic in human populations exposed to malaria (35, 41); it is conceivable that it is advantageous to the parasite to evoke an antibody response to this part of MSP-1, and this may provide a selective pressure to prevent sequence variation in the conserved parts of the molecule.

The physiological function of the proteolytic processing of MSP-1, and the identity of the protease which mediates it, are unknown. However, these results reemphasize the importance of the processing step, and the potential of the relevant enzyme as a novel target for development of protease inhibitor–based antimalarial drugs.

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References

1. Cohen, S., I.A. McGregor, and S.C. Carrington. 1961. Gamma globulin and acquired immunity to human malaria. Nature (Lond.). 192:733–737.
2. Edozien, J.C., H.M. Gilles, and I.O.K. Udowo. 1962. Adult and cord-blood gamma-globulin and immunity to malaria in Nigeria. Lancet. 2:951–955.
3. McGregor, I.A., S.P. Carrington, and S. Cohen. 1963. Treatment of East African Plasmodium malaria with West African human gamma globulin. Trans. R. Soc. Trop. Med. Hyg. 57:170–175.
4. Bouharoun-Tayoun, H., P. Attanath, A. Sabchareon, T. Chongsuphajisiddhi, and P. Drulilie. 1990. Antibodies that protect humans against Plasmodium falcerum blood stages do not on their own inhibit parasite growth and invasion in vitro, but act in cooperation with monococytes. J. Exp. Med. 172:1633–1641.
5. Holder, A.A. 1994. Proteins on the surface of the malaria parasite and cell invasion. Parasitolology. 108:S5–S18.
6. Barnwell, J.W., and M.R. Galinski. 1991. The adhesion of malaria merozoite proteins to erythrocytes: a reflection of function? Res. Immunol. 142:666–672.
7. McBride, J.S., and H.-G. Heidrich. 1987. Fragments of the polymorphic M, 185,000-glycoprotein from the surface of isolated Plasmodium falcerum merozoites form an antigenic complex. Mol. Biochem. Parasitol. 30:S1–S18.
8. Holder, A.A., J.S. Sandhu, Y. Hillman, L.S. Davey, S.C. Nicholls, H. Cooper, and M.J. Lockyer. 1987. Processing of the precursor to the major merozoite antigens of Plasmodium falcerum. Parasitolology. 94:199–208.
9. Blackman, M.J., H.-G. Heidrich, S. Donachie, J.S. McBride, and A.A. Holder. 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.
10. Blackman, M. J., H. W. Hittle, and A. A. Holder. 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.
11. Blackman, M. J., and A. A. Holder. 1992. Secondary processing of the Plasmodium falciparum merozoite surface protein–1 (M SP1) by a calcium-dependent membrane-bound serine protease: shedding of M SP1 as a noncovalently associated complex with other fragments of the M SP1. Mol. Biochem. Parasitol. 50:307–316.
12. Stafford, W. H. L., M. J. Blackman, A. Harris, S. Shai, M. Grainger, and A. A. Holder. 1994. N-terminal amino acid sequence of the Plasmodium falciparum merozoite surface protein–1 (M SP-1) polypeptides. Mol. Biochem. Parasitol. 66:157–160.
13. Blackman, M. J., J. A. Chappel, S. Shai, and A. A. Holder. 1993. A conserved parasite serine protease processes the Plasmodium falciparum merozoite surface protein–1 in vivo and in vitro. Mol. Biochem. Parasitol. 72:111–119.
14. Blackman, M. J., E. D. Dennis, E. M. A. Hirst, C. H. Kocken, T. J. Scott-Finnigan, and A. W. T. Thomas. 1996. Plasmodium knowlesi: secondary processing of the malaria merozoite surface protein–1. Exp. Parasitol. 83:229–239.
15. M. C. Ean, P. G., K. O’Dea, and K. N. Brown. 1993. A single amino acid determines the specificity of a monoclonal antibody which inhibits Plasmodium falciparum AS in vivo. Mol. Biochem. Parasitol. 62:211–222.
16. M. Quinlan, W. R., T. M. Daly, W. P. W. Eidiand, and C. A. Long. 1984. Passive immunization against murine malaria with an IgG3 monoclonal antibody. J. Immunol. 132:3131–3137.
17. Ling, I. T., S. Ogure, and A. A. Holder. 1993. Immunization against malaria with a recombinant protein. Parasite Immunol. (Oxf.). 16:63–67.
18. Daly, T. M., and C. A. Long. 1993. A recombinant 15-kilodalton carboxyl-terminal fragment of Plasmodium yoelii yoelii 17X L merozoite surface protein 1 induces a protective immune response in mice. Infect. Immun. 61:2462–2467.
19. Tian, J.-H., L. H. Miller, D. C. Kaslow, J. Ahlers, M. F. Good, D. W. Alling, J. A. Berzofsky, and S. Kumar. 1996. Genetic regulation of protective immune response in congenic strains of mice vaccinated with a subunit malaria vaccine. J. Immunol. 125:1176–1183.
20. Hui, G. S. N., and W. A. Siddiqui. 1987. Serum from Pf195 protected Aotus monkeys inhibit Plasmodium falciparum growth in vitro. Exp. Parasitol. 64:519–522.
21. Chang, S. P., H. L. Gibson, C. T. Lee-Ng, P. J. Barr, and G. S. N. Hui. 1992. A carboxy-terminal fragment of Plasmodium falciparum gp195 expressed by a recombinant baculovirus induces antibodies that completely inhibit parasite growth. J. Immunol. 149:548–555.
22. Pirson, P. J., and M. E. Perkins. 1985. Characterization with monoclonal antibodies of a surface antigen of Plasmodium falciparum merozoites. J. Immunol. 134:1946–1951.
23. Cooper, J. A., L. T. Cooper, and A. J. Saul. 1992. Mapping of the region predominantly recognized by antibodies to the Plasmodium falciparum merozoite surface antigen MSA 1. Mol. Biochem. Parasitol. 51:301–312.
24. Blackman, M. J., T. J. Scott-Finnigan, S. Shai, and A. A. Holder. 1994. Antibodies inhibit the protease-mediated processing of a malaria merozoite surface protein. J. Exp. Med. 180:389–393.
25. Burghaus, P., and A. A. Holder. 1994. Expression of the 19-kilodalton carboxy-terminal fragment of the Plasmodium falciparum merozoite surface protein–1 in Escherichia coli produces a correctly folded protein that is recognized by protective monoclonal antibodies. Mol. Biochem. Parasitol. 64:165–169.
26. Smith, D. B., and K. S. Johnson. 1988. Single-step purification of polypeptides expressed in E. coli as fusions with glutathione S-transferase. Gene (Amst.). 67:31–40.
27. Abath, F. G. C., and A. J. G. Simpson. 1990. A simple method for the recovery of purified recombinant peptides cleaved from glutathione S-transferase fusion proteins. Protein Res. 3:167–168.
28. Miller, L. H., T. Roberts, M. Shahabuddin, and T. F. M. C. Cutchan. 1993. Analysis of sequence diversity in the Plasmodium falciparum merozoite surface protein–1 (M SP-1). Mol. Biochem. Parasitol. 59:1–14.
29. Shears, E., P. C. Uetreccas, and H. B. Pollard. 1971. The purification of β-galactosidase from E. coli by affinity chromatography. J. Biol. Chem. 246:196–200.
30. Fraker, J. P., and J. C. Speck. 1978. Protein and cell membrane iodination with a sparingly soluble chloramide 1,3,4,6-tetrachloro-3,6-diphenylglycoluril. Biochem. Biophys. Res. Commun. 80:849–857.
31. Blackman, M. J. 1994. Purification of Plasmodium falciparum merozoites for analysis of the processing of merozoite surface protein–1. Methods Cell Biol. 45:213–220.
32. Blackman, M. J., and A. A. Holder. 1993. Use of a recombinant baculovirus product to measure naturally-acquired human antibodies to disulphide-constrained epitopes on the P. falciparum merozoite surface protein–1 (M SP1). FEMS Immunol. Med. Microbiol. 6:307–316.
33. Blackman, M. J., I. T. Ling, S. C. Nichols, and A. A. Holder. 1991. Proteolytic processing of the Plasmodium falciparum merozoite surface protein–1 produces a membrane-bound fragment containing two epidermal growth factor–like domains. Mol. Biochem. Parasitol. 49:29–34.
34. Chappel, J. A., and A. A. Holder. 1993. Monoclonal antibodies that inhibit Plasmodium falciparum invasion in vitro recognize the first growth factor–like domain of merozoite surface protein–1. Mol. Biochem. Parasitol. 60:303–312.
35. Wilson, C. F., R. Anand, J. T. Clark, and J. S. McBride. 1987. Topography of epitopes on a polymorphic antigen of Plasmodium falciparum determined by the binding of monoclonal antibodies in a two-site radioimmunoassay. Parasite Immunol. (Oxf.). 9:737–746.
36. Dvorák, J. A., L. H. Miller, W. C. Whitehouse, and T. Shiroti. 1975. Invasion of erythrocytes by malaria merozoites Science (Wash. D.C.). 187:748–750.
37. Stafford, W. H., B. Günder, A. Harris, H. -G. Heidrich, A. A. Holder, and M. J. Blackman. 1996. A 22 kDa protein associated with the Plasmodium falciparum merozoite surface protein–1 complex. Mol. Biochem. Parasitol. 80:159–169.
38. Riley, E. M., S. J. Allen, J. G. Wheeler, M. J. Blackman, S. Bennett, B. Takacs, H. -J. Schonfelds, A. A. Holder, and B. M.
Greenwood. 1992. Naturally acquired cellular and humoral immune responses to the major merozoite surface antigen of Plasmodium falciparum are associated with reduced malaria morbidity. Parasite Immunol. (Oxf.). 14:321–337.

42. Egan, A.F., J. Morris, G. Barnish, S. Allen, B.M. Greenwood, D.C. Kaslow, A.A. Holder, and E.M. Riley. 1996. Clinical immunity to Plasmodium falciparum malaria is associated with serum antibodies to the 19-kDa C-terminal fragment of the merozoite surface antigen, PfMSP-1. Parasitol. Today. 13:46–47.

43. Chappel, J.A., A.F. Egan, E.M. Riley, P. Druilhe, and A.A. Holder. 1994. Naturally acquired human antibodies which recognize the first epidermal growth factor–like module in the Plasmodium falciparum merozoite surface protein 1 do not inhibit parasite growth in vitro. Infet. Immun. 62:4488–4494.

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

45. Miller, L.H., M.F. Good, and D.C. Kaslow. 1997. The need for assays predictive of protection in development of malaria bloodstage vaccines. Parasitol. Today. 13:46–47.

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

47. Chang, S.P., S.E. Case, W.L. Gosnell, A. Hashimoto, K.J. Kramer, L.Q. Tam, C.Q. Hashiro, C.M. Nakado, H.L. Gibson, C.T. Lee-Ng et al. 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.

48. Tanabe, K., M. Mackay, M. Goman, and J.G. Scaife. 1987. Allelic dimorphism in a surface antigen gene of the malaria parasite Plasmodium falciparum. J. Mol. Biol. 195:273–287.