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# Human Antibody Response to *Plasmodium falciparum* Merozoite Surface Protein 2 Is Serogroup Specific and Predominantly of the Immunoglobulin G3 Subclass

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**MSP2 is a merozoite surface protein of *Plasmodium falciparum* and, as such, is a potential component of a malaria vaccine. In this study, we have used a panel of recombinant MSP2 antigens in enzyme-linked immunosorbent assays to investigate the recognition of MSP2 by antibodies from malaria-immune human serum. These recombinant antigens include full-length proteins of serogroups A and B and fragments representing the conserved, group-specific, or repeat regions of each serogroup. Ninety-five percent of the serum samples tested contained MSP2-specific antibodies: 81% of serum samples tested responded to serogroup A, and 86% responded to serogroup B. The antibody response is directed almost exclusively towards dimorphic and polymorphic regions of MSP2; the conserved regions are rarely recognized, and antibodies to serogroups A and B do not cross-react. Interestingly, the antibody response is predominately of the cytophilic and complement-fixing subclass immunoglobulin G3.**

Antigens on the surface of malaria merozoites are of interest as potential targets for vaccine-induced immune responses. Since these antigens may be involved in merozoite adherence to, and invasion of, erythrocytes (17), antibodies specific for merozoite surface antigens could prevent invasion of erythrocytes and thereby interrupt the asexual cycle of parasite proliferation.

One such antigen, of the human malaria parasite *Plasmodium falciparum*, is the merozoite surface protein 2 (MSP2; also called MSA2, gp35-56, or GP3). Several pieces of evidence suggest that antibodies to MSP2 may be involved in protective immunity to malaria. Monoclonal antibodies (MAbs) to MSP2 have been shown to inhibit parasite growth in vitro (5, 10), MSP2 is among the antigens recognized by antibodies that inhibit merozoite dispersal (34), and mice immunized with peptides corresponding to the conserved regions of MSP2 of *P. falciparum* are protected against challenge with the rodent parasite *Plasmodium chabaudi* (27).

Sequencing data and serological characterization of malaria parasites with MSP2-specific MAbs (12, 29–31, 34) show that MSP2 is a polymorphic protein which can be classified into two allelic families corresponding to two antigenically distinct serogroups. Serogroup A includes the strains T9/96, Camp, 3D7, and IC1, and serogroup B includes the strains FCQ-27, K1, and Dd2. The protein has conserved sequences, at the amino and carboxy termini, which are common to both serogroups. These conserved regions flank dimorphic, group-specific sequences containing two central regions of polymorphic, tandemly arranged repetitive sequences. Fenton et al. (12) designated the two regions of tandem repeats R1 and R2. The R1 region of serogroup A is glycine, serine, and alanine rich and varies considerably from isolate to isolate; the R2 region is a relatively conserved repeat, rich in the amino acid threonine. The repeat sequences of serogroup B are unrelated to those of serogroup A and consist of either 32 amino acids (R1) or 12 amino acids (R2). Although the sequences of group B repeats are relatively conserved between isolates, they vary in number.

The extensive sequence polymorphism of MSP2 raises the important question of whether immune responses to the protein might be sequence specific. If this is the case, then responses to MSP2 may be implicated in the notion that the slow development of protective immunity to malaria, in people living in areas where malaria is endemic, is due to the gradual acquisition of specific immunity to all of the variant genotypes of the parasite circulating in the community (23). To better understand the nature of protective immunity to malaria, it is necessary to determine whether naturally acquired antibody responses to MSP2 are cross-reactive within and between serogroups. Such information would also be useful for determining which, if any, regions of MSP2 might usefully be included in a malaria vaccine. To date, there is very little published data on naturally acquired immunity to MSP2 and no information on the relative immunogenicity of conserved group-specific (dimorphic) and repetitive (polymorphic) regions of the molecule. A study of adult, malaria-exposed, Melanesians showed that 82% had antibodies against MSP2 serogroup B (26), but the specificity of these antibodies was not fully characterized. A recent study in an area of high endemicity of Papua New Guinea found high antibody prevalence (≥90%) to recombinant antigens representing the full-length proteins of both MSP2 serogroup A (3D7) and serogroup B (FC27) (1). Using a serogroup A construct lacking the central repeats, this group also showed that a proportion of immune individuals responded only to epitopes within the repeat region. Experimental immunization of *Aotus* monkeys with *P. falciparum* results in an anti-MSP2 antibody response directed primarily to repeat and group-specific regions of MSP2 (34). Antibodies purified from immune clusters of merozoites also recognize

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group-specific sequences, suggesting that this region is accessible at the surface of the intact parasite and may therefore be a target for parasite-inhibitory immune responses (34). However, immunoblotting studies with affinity-purified human serum suggest that naturally immunodominant epitopes are encoded within the repetitive sequences of the molecule (31). None of these studies has addressed the crucial question of whether sequence polymorphism gives rise to antigenic diversity and whether epitopes in one allelic form of the protein cross-react with similar epitopes from other parasites.

Here we report a comprehensive study of the recognition of MSP2 by sera from malaria-immune adults from The Gambia, West Africa, and the identification of regions of the molecule that are immunogenic during malaria infections. We find that MSP2 is naturally immunogenic in man, inducing immunoglobulin G (IgG) antibodies which predominantly recognize epitopes located in the dimorphic and polymorphic regions of the molecule. Importantly, antibodies to the two main serogroups of MSP2 (A and B) do not appear to cross-react. Anti-MSP2 antibodies were found to belong mainly to IgG subclasses that have opsonizing and complement-fixing properties, suggesting that they might play a role in regulating the proliferation of intraerythrocytic parasites.

## MATERIALS AND METHODS

**Sera.** Serum samples were obtained from 70 adults (aged 15 to 65 years) living in rural and periurban areas of The Gambia, West Africa, where malaria transmission is intense during the short wet season (from July to November) and minimal at other times of the year (15). Control serum samples were obtained from 15 European adults who had not been exposed to malaria.

**Antigens. (i) Recombinant MSP2 proteins.** pGEX expression vectors were used to direct the synthesis of MSP2 polypeptides in *Escherichia coli* as fusions with the C terminus of glutathione *S*-transferase (GST) of *Schistosoma japonicum* (27a, 28). This permits the purification, on a glutathione column, of recombinant antigens as stable, soluble fusion polypeptides. Expression of the protein was initiated by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to cultures of transformed *E. coli*. After 3 to 5 h, cells were pelleted, resuspended in phosphate-buffered saline (PBS), and lysed on ice by mild sonication in the presence of 10% (vol/vol) Triton X-100 (BDH Chemicals, Poole, United Kingdom). The fusion proteins were purified from the supernatant by absorption onto glutathione agarose beads (Sigma, Poole, United Kingdom). The proteins were eluted by competition with free glutathione, and their concentrations were estimated by the intensity of Coomassie blue staining on acrylamide gels.

As a control, the fusion protein partner, GST, was purified from pGEX plasmids lacking an MSP2 insert.

The immunogenicity and antigenic integrity of the fusion proteins were assessed by mouse immunization and testing of the mouse sera in an enzyme-linked immunosorbent assay (ELISA), by Western blotting (immunoblotting), and by immunofluoresence assays (with acetone-fixed, mature *P. falciparum* schizonts) (27a). All of the recombinant proteins used in this study were shown to reflect the antigenic character of the native protein and are represented in Fig. 1. It has not yet been possible to produce a recombinant protein which accurately reflects the antigenic structure of the N-terminal conserved sequence of the protein; hence, no analysis of responses to this region is reported.

**(ii) MSP1-p190.1.** As a positive control for prior exposure to *P. falciparum*, sera were also tested for reactivity with a conserved sequence from another merozoite surface protein, MSP1. p190.1 represents a nonvariable region of MSP1 (gp190) which has previously been shown to be recognized by more than 90% of malaria-exposed individuals (25) and was produced in *E. coli* as a free polypeptide (13). p190.1 was a kind gift of J. R. L. Pink, F. Hoffman La Roche, Basel, Switzerland.

**ELISA.** Microtiter plates (Immulon-4; Dynatech) were coated overnight at 4°C with 100 μl of antigen per well at 0.5 μg/ml in 0.1 M carbonate ($Na_2CO_3$-$NaHCO_3$) buffer (pH 9.6) and blocked for 5 h at room temperature with 200 μl of blocking buffer (1% [wt/vol] milk powder in PBS–0.05% Tween 20) per well. At the same time, sera were diluted in blocking buffer and incubated at room temperature for 5 h to maximize absorption of nonspecific antibodies. Plates were washed three times with PBS-Tween, 100 μl of diluted serum per well was added to duplicate wells, and the plates were incubated overnight at 4°C. The plates were washed and incubated with horseradish peroxidase-conjugated rabbit anti-human IgG antibody (Dako Ltd., High Wycombe, United Kingdom) for 3 h at room temperature. For the detection of specific IgG subclasses, the plates were incubated for 3 h with murine MAbs to specific human IgG subclasses, followed by horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin antibody for 3 h. The subclass specificity of these MAbs (IgG1 [NL16; Boehringer GmbH, Mannheim, Germany], IgG2 [ROM1; Boehringer], IgG3 [HP6050; Serotec, Oxford, England], IgG4 [RJ4; Boehringer]) has been widely reported (8, 19–21) and was reconfirmed before their use in this study. Each MAb was titrated against various concentrations of purified IgG of the appropriate subclass; all MAbs gave parallel titration curves. For each MAb, a working concentration was selected such that the relationship between optical density (OD) and IgG concentration was approximately the same for each subclass. This allows the amounts of antibody in each subclass to be assessed, on a roughly quantitative basis, by comparing the OD values.

All plates were developed with $H_2O_2$ as the substrate and *o*-phenylenediamine as the chromagen at 4°C, and the reaction was stopped after 10 min with 20 μl of 2 M $H_2SO_4$ per well. The OD was measured at a wavelength of 492 nm.

Optimal concentrations of antigen and antibody were determined by checkerboard titrations.

**Statistical analysis.** The reactivity of the sera with various MSP2 fusion proteins in an ELISA was calculated by subtracting the OD value for the GST control from the value obtained for the MSP2 fusion protein to obtain specific OD values. Positive samples were defined as those giving a specific OD above the normal range for control European sera. The normal range was taken as the mean ± 2 standard deviations of 15 control serum samples.

The reactivities of individual serum samples with different antigens were compared by means of Spearman's rank correlation test (32).

## RESULTS

**Reactivity of human serum antibodies with MSP2 fusion proteins.** Serum IgG antibodies from Gambian and European adults were tested for recognition of recombinant MSP2 proteins in an ELISA. MSP2 proteins were specifically recognized by sera from individuals who had been exposed to malaria (Fig. 2). The cutoff level for positive sera for each antigen ranged from 0.117 to 0.384 OD units (Fig. 2). The proportion of sera recognizing each of the proteins is shown in Table 1.

These results show that MSP2 is well recognized by sera from individuals who have been exposed to malaria. Serogroup A and serogroup B are recognized by 81 and 86% of serum samples, respectively, indicating that MSP2 is highly antigenic during natural infections. MSP2-specific antibodies predominantly recognize polymorphic and dimorphic regions of the protein; the conserved C terminus is recognized by only 36% of serum samples, and the OD values obtained with this protein were significantly lower than those for the full-length and group-specific proteins. Only three serum samples recognized this protein with an OD value of greater than 0.300, and given that the normal range for European sera may underestimate the normal range for African sera (which have higher total IgG concentrations than European sera and thus probably give higher backgrounds), these three serum samples are possibly the only ones which truly contain antibody to the C terminus.

**Lack of antigenic cross-reactivity between serogroups A and B.** Seventy-nine percent of serum samples from adult Gambians recognized both the A and B serogroups (proteins A1 and B1, which represent the full-length molecules). To determine whether this dual recognition was due to a single population of antibodies which react with epitopes common to both proteins or whether the serum contained two separate, non-cross-reacting populations of antibody, we compared the reactivities of individual sera to the full-length proteins from both serogroups (A1 and B1) (Fig. 3). It is clear that although some sera recognize the two proteins apparently equally, other sera clearly recognize one protein but not the other. Comparisons of A2 with B2 and A3 with B3 gave essentially identical results (data not shown).

By use of Spearman's rank correlation test, a positive correlation was obtained for responses to A1 and B1 ($r = 0.555$; $r_s$ [Spearman's rank correlation coefficient] = 0.307, $P = 0.005$). However, we consider the high level of statistical significance to be the result of the large sample size and the considerable number of double-negative sera; the association is in fact quite weak and may simply reflect independent ex-

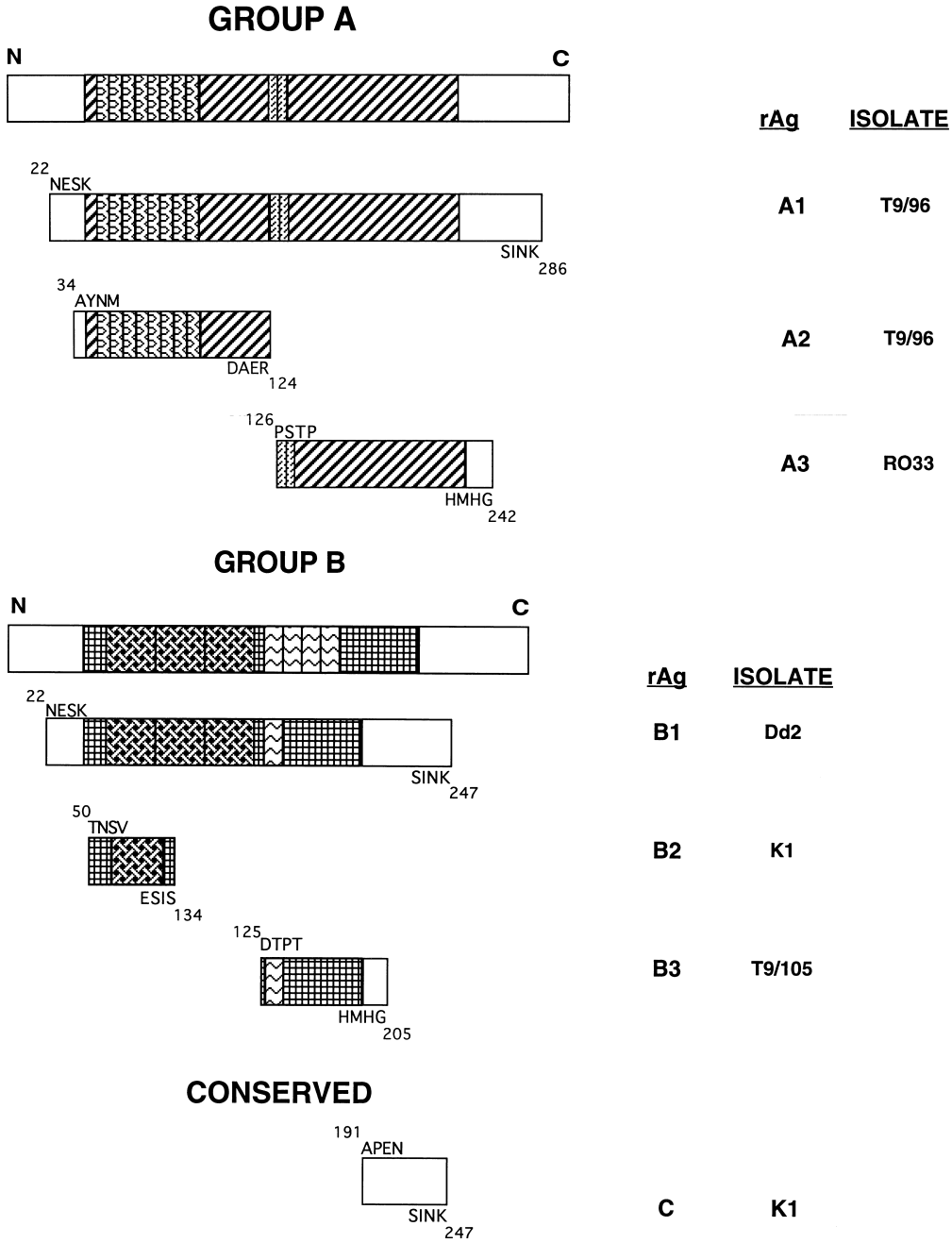


FIG. 1. Schematic representation of the MSP2 fusion proteins. The isolates from which the recombinant proteins were derived are indicated in bold type. The amino acid sequences at the start and end of each recombinant antigen are given. The amino acid numbers refer to the positions of the first and last residues of the recombinant antigens from the published sequences of T9/96 (serogroup A) and FC27 (serogroup B) (12). A1 to A3, B1 to B3, and C are the codes for the recombinant antigens. A1 and B1 represent almost full-length proteins of MSP2 serogroups A and B, respectively. A2 and B2 represent the R1 repeat region of each serogroup, and A3 and B3 represent the predominantly group-specific region in the C-terminal half of the molecule. C represents the conserved C terminus of MSP2. Symbols: ▒, A R1 repeats; ▓, B R1 repeats; ▒, A R2 repeats; ▒, B R2 repeats; ▨, group A specific; ▦, group B specific; □, conserved.

posure to both serogroups of *P. falciparum*. As an estimate of association due to exposure, we compared the recognition of MSP2 proteins with the recognition of a recombinant protein representing a conserved region of an unrelated merozoite surface protein (MSP1, p190.1). Correlation coefficients of up to 0.492 were obtained. Thus, the correlation coefficient obtained for A1 versus B1 was only slightly higher than that obtained for MSP2 versus MSP1, suggesting that the correlation is indeed due to exposure rather than to cross-reacting antibodies.

To confirm that antibodies to the two serogroups are not cross-reactive, individual sera were tested in competition ELISAs. Sera which were known to contain antibodies to both A1 and B1 were selected; these sera were preincubated with either serogroup A1 or B1 and tested in an ELISA for recognition of the other protein. The example shown in Fig. 4 was

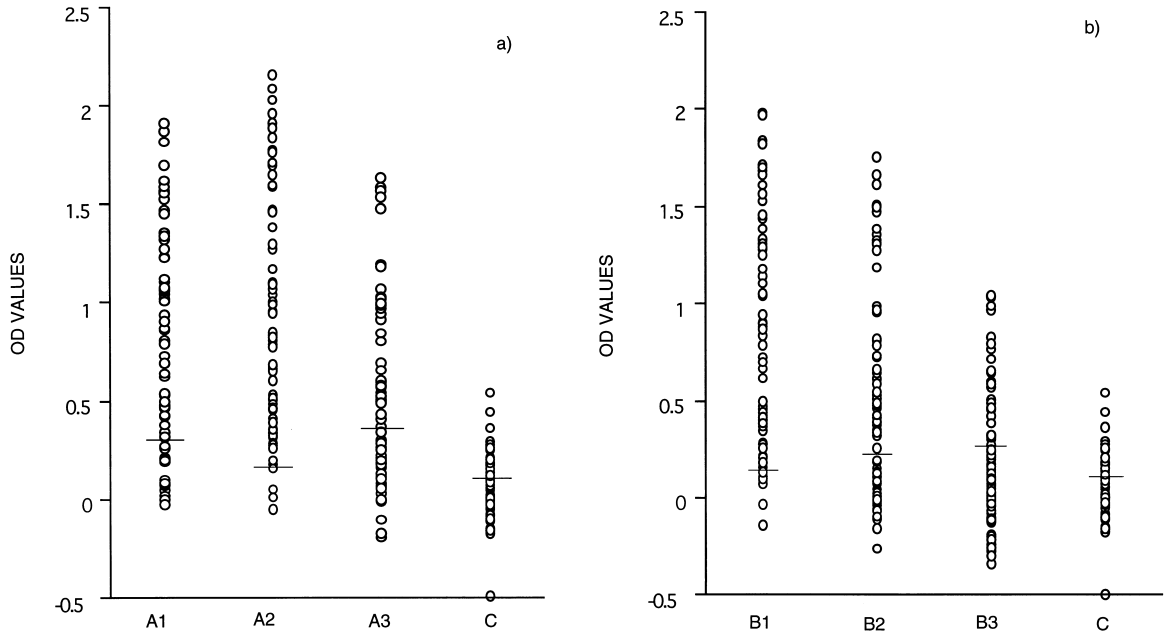


FIG. 2. Dot plots showing specific IgG responses (OD at 492 nm) to MSP2 fusion proteins of serogroups A (a) and B (b) in 70 Gambian serum samples. The cutoff level for positive sera is indicated by a horizontal line. Sera were tested at a dilution of 1/1,000.

typical of the many sera tested: while preincubation with increasing concentrations of A1 prevents subsequent binding of antibodies to A1-coated plates, it has no effect on binding of antibody to B1-coated plates and vice versa. Thus, in double-positive sera, there appear to be two distinct populations of antibodies, one specific for serogroup A and the other specific for serogroup B.

**Subclass of anti-MSP2 IgG antibodies.** The IgG subclass of anti-MSP2 antibodies was determined for sera that had been shown to contain MSP2-specific IgG. Figure 5 shows the IgG subclasses of antibodies to proteins A1, A2, and A3. The distribution was similar for the corresponding serogroup B proteins (data not shown).

Although the IgG subclass pattern differs slightly for antibodies to the different proteins (Fig. 5), the predominant MSP2-specific antibody subclass is IgG3. Seventy-seven and 80%, respectively, of A1- and A3-positive serum samples contained only IgG3. The remainder of the A1- and A3-positive sera contained only IgG1. In comparison, 44% of A2-positive serum samples contained A2-specific IgG4. A2 is a fragment of the A1 construct; therefore, one would have expected some IgG4-containing A1-positive sera. However, the majority of the A2 IgG4-positive results were close to the cutoff value and gave values below the cutoff for A1 IgG4. The two serum samples which were strongly positive for A2 IgG4 were not tested against A1 since they did not emerge as antibody positive for A1 IgG.

The pattern of recognition is similar for the serogroup B proteins in that IgG3 is the predominant subclass. However,

TABLE 1. Percentage of malaria-immune serum samples recognizing recombinant MSP2 proteins[a]

| Serogroup | % Responders[b] | OD value range[c] | |
|---|---|---|---|
| | | Min | Max |
| A1 | 81 | −0.026 | 1.911 |
| A2 | 81 | −0.053 | 2.157 |
| A3 | 73 | −0.194 | 1.632 |
| B1 | 86 | −0.140 | 1.981 |
| B2 | 64 | −0.256 | 1.755 |
| B3 | 43 | −0.339 | 1.044 |
| C (conserved) | 36 | −0.493 | 0.542 |

[a] Seventy Gambian serum samples were tested in an ELISA for recognition of MSP2 proteins.

[b] The percentage of responders was calculated as those serum samples giving OD values greater than the mean + 2 standard deviations of the OD values obtained for 15 European (malaria nonexposed) serum samples (see Fig. 2).

[c] The range of OD values (minimum and maximum) obtained for each protein are shown. (Negative values occur when the OD value of the GST control protein exceeds the OD value of the fusion protein.)

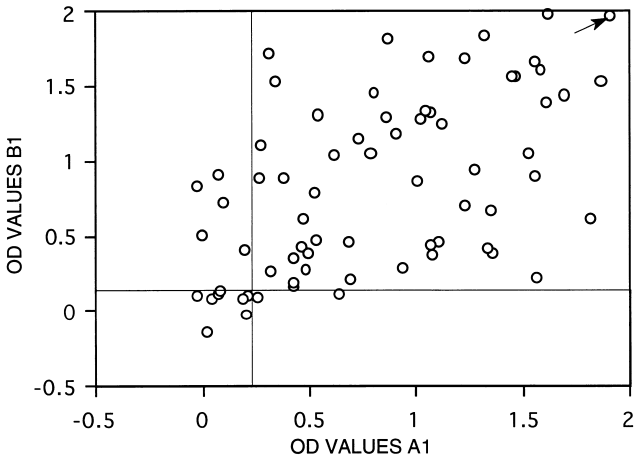


FIG. 3. Comparison of OD values for serogroups A1 and B1 for serum samples from 70 Gambian adults. Solid lines indicate the cutoff level as determined from the mean + 2 standard deviations of 15 European serum samples. The arrow indicates the serum sample shown in the competition ELISA (Fig. 4). Each circle represents a single serum sample tested at a dilution of 1/1,000.

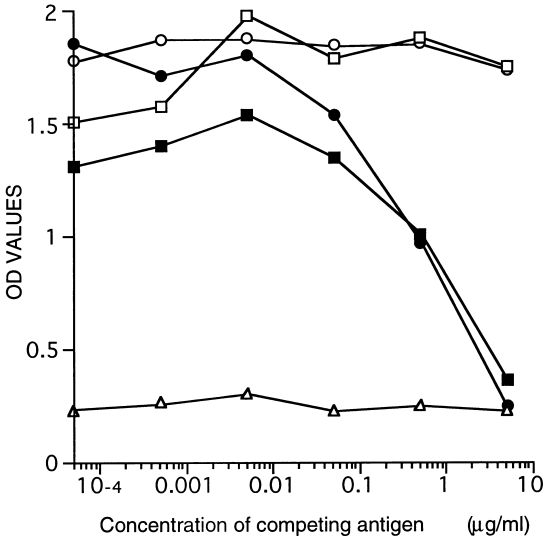


FIG. 4. Competition assay to determine the extent of cross-reactivity between antibodies recognizing A1 and B1. The results for a single, typical serum sample are shown. Sera were preincubated with antigen at concentrations from 0 to 5 μg/ml. Sera were tested at a dilution of 1/1,000. Symbols: ●, A1 on plate versus A1 in serum; ○, A1 versus B1; ■, B1 versus B1; □, B1 versus A1; △, GST versus GST.

approximately 57% of serogroup B-positive serum samples were IgG1 positive; in the majority of cases, IgG1 was coexpressed with IgG3.

This predilection for induction of IgG3 antibodies is unusual and suggests that there may be something about the antigenic structure of the MSP2 molecule which preferentially triggers isotype switching to IgG3 in MSP2-specific plasma cells.

## DISCUSSION

The aim of this study was to characterize the reactivity of serum antibodies from malaria-exposed adult individuals with the merozoite surface protein MSP2. Evaluation of MSP2 as a potential component of a subunit malaria vaccine requires an understanding of the naturally occurring immune response to MSP2 and, most importantly, the immunological significance of amino acid sequence polymorphisms. In particular, it is important to determine whether antibodies against one MSP2 serogroup will cross-react with the other serogroup or whether the antibody response is group specific or allele specific. Such information may also help us to understand the relative importance of allele-specific (strain-specific) immune responses in the acquisition of clinically protective immunity to malaria by people living in areas where malaria is endemic. There are two main theories to explain the slow development of protective immunity to malaria which is typically seen in individuals living in areas of endemicity: (i) polymorphism of antigens which are the targets of protective immune responses, and (ii) intrinsically poor immunogenicity of the target molecules. The data presented here indicate that the latter explanation is not true, at least with respect to MSP2. Sixty-seven of the 70 individuals tested had clearly detectable anti-MSP2 antibodies (to either serogroup A, serogroup B, or both) with endpoint titrations of >1/9,000 for most serum samples (data not shown). It is possible that the three seronegative individuals had not been exposed to parasites carrying the MSP2 variants tested here. In addition, we have screened a small number of serum samples collected 2 to 4 weeks after a known primary

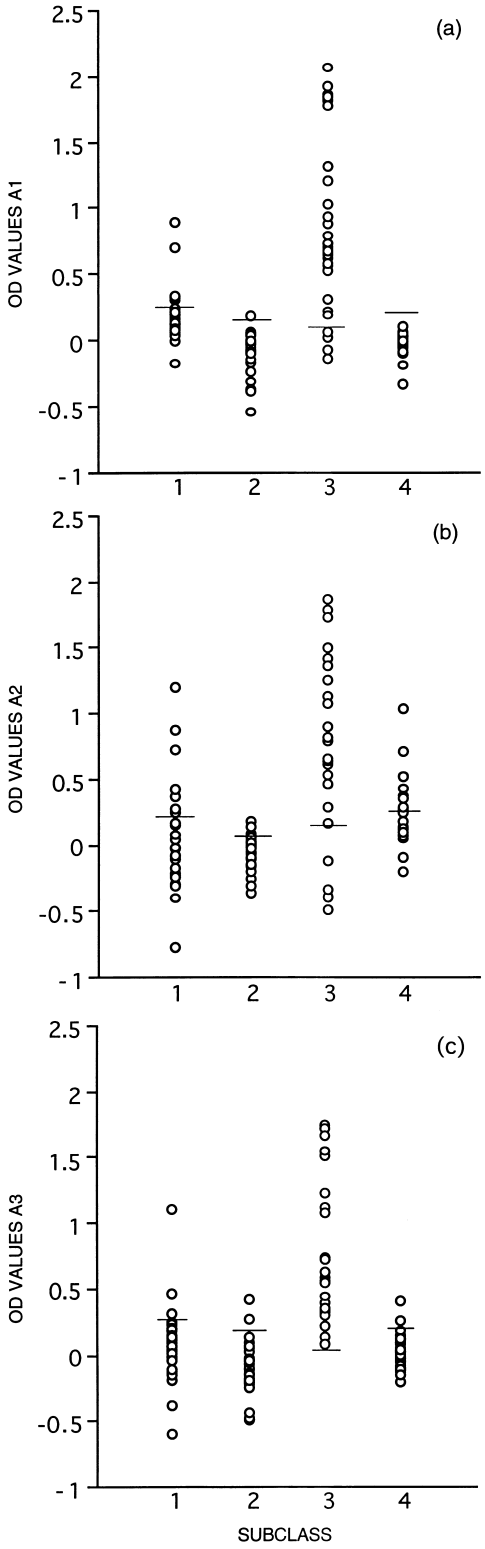


FIG. 5. Dot plots of IgG subclass of antibodies to serogroup A proteins. (a) A1 ($n$ = 30); (b) A2 ($n$ = 25); (c) A3 ($n$ = 30). Horizontal lines represent the mean + 2 standard deviations of 15 control serum samples.

malaria infection, and all contained detectable levels of antibody to MSP2 (33b).

Antibodies tended to recognize epitopes within the dimorphic and polymorphic regions of MSP2; the conserved C terminus seems to be poorly antigenic. This is in agreement with the findings of Thomas et al. (34), who reported that although intact MSP2 is recognized by monkey and human antibodies eluted from intact merozoites, such antibodies did not recognize peptides representing the N- and C-terminal regions of the molecule. Similarly, Saul et al. (27) were unable to detect antibodies against synthetic peptides representing the conserved regions of MSP2 in sera from 18 people with high titers of anti-parasite antibodies (as determined by immunofluorescence assays).

Importantly, the sequences which are conserved within each allelic family (detected by use of A3 and B3 proteins) are highly immunogenic. Ninety percent of the serum samples tested contained antibodies which recognize either A3 or B3 (or both); thus, a vaccine based on MSP2 may need to contain only two different antigens, representing the group-specific sequences of each serogroup. Interestingly, the group-specific A3 protein is recognized by significantly more individuals than the B3 protein. This may reflect more frequent or more recent exposure of the donors to parasites of serogroup A since approximately 60% of parasites isolated in The Gambia belong to the A serogroup (6, 33a). Antibodies eluted from immune clusters of merozoites recognize dimorphic MSP2 sequences (34), indicating that epitopes associated with these sequences are accessible at the surface of intact merozoites and may therefore be a target for inhibitory antibodies.

Proteins which represent the polymorphic R1 repeat regions of both serogroups (i.e., A2 and B2) were recognized by a substantial proportion of the sera, a greater proportion in fact than that which recognized the dimorphic regions. This finding was somewhat unexpected since the sera were tested against only one variant for each serogroup, and at least for serogroup A, the amino acid sequence of the repeats from different isolates varies extensively (22, 24). One likely explanation of this finding is that the antibodies detected were in fact recognizing epitopes within a short N-terminal segment of group-specific sequence contained in the protein A2 (Fig. 1). Alternative explanations include extensive immunological cross-reactivity between different repeat sequences or a very high frequency of parasites in The Gambia expressing the tested R1 repeat sequence. This latter explanation is unlikely since considerable microheterogeneity within repetitive sequences has been reported for parasite populations and isolates from all over the world (7, 12, 22, 24, 30, 31, 33a). Immunological cross-reactivity may well occur among the R1 repeats of serogroup B, where amino acid sequences are relatively conserved, but is less likely for serogroup A. Further studies are underway to study the effect, on antibody recognition, of variation in the sequence and number of repeats.

Quantification of absolute amounts of antibody by an ELISA is difficult since the OD value obtained is dependent on both the concentration of antibody and its affinity. It is not possible to compare the amounts of antibody in sera which react with different recombinant antigens, since the number of epitopes in each assay (and thus the avidity of the reaction) is unknown (35). Therefore, to make comparisons between the antibody responses to different proteins, we used a nonparametric rank correlation test to compare specific OD values for individual sera, tested at a single dilution, against the full-length proteins of serogroup A and serogroup B. Such comparisons show that the correlation between responses to the two serogroups is weak and probably due to exposure to parasites of both serogroups rather than to cross-reactive antibodies. Competition experiments clearly demonstrate that antibodies to serogroup A and B are not cross-reactive, since protein A1 cannot compete for binding of B1-specific antibodies and vice versa.

In theory, antibodies specific for MSP2 could inhibit merozoite invasion of erythrocytes by a number of mechanisms, including merozoite agglutination, complement-mediated lysis, opsonization, or blocking of receptors involved in the adherence of merozoites to erythrocytes. These various mechanisms would be mediated by antibodies of different IgG subclasses; only IgG1 and IgG3 are opsonizing and complement fixing, but all four subclasses could mediate agglutination or receptor blocking. Since functional differences may exist among antibodies of the same specificity, we have determined the subclass of MSP2-specific IgG in sera from individuals immunized by natural exposure to malaria. Our work indicates that antibodies which recognize MSP2 are predominantly of the IgG3 subclass. It has been suggested that the ratio of IgG1 and IgG3 to IgG2 and IgG4 may be important in immunity to asexual blood stages of *P. falciparum* (2, 16) since, while IgG1 and IgG3 can mediate opsonization and phagocytosis of parasitized erythrocytes or free merozoites, IgG2 and IgG4 antibodies (of the same epitope specificity) may block the binding of the protective subclasses. IgG3 is considered to be the most effective subclass for activating the complement pathway (4), and it is known to mediate cell lysis by monocytes or Fc-receptor-bearing lymphocytes (33). Thus, the predominance of the IgG3 response to MSP2 in adults with a high degree of protective immunity to malaria suggests that this molecule may be a target of protective antibody responses.

The predominance of the IgG3 response is unusual and noticeably different from the response to MSP1 (9). The only other examples, of which we are aware, of an antibody response which is significantly skewed towards IgG3 are the response to the outer membrane protein of *Branhamella catarrhalis* in which IgG3 antibodies represent approximately 70% of the total response in individuals over 4 years of age (14) and responses to the streptolysin M protein in which IgG3 predominated in the response of more than half of the individuals tested (11). Interestingly, in the case of *B. catarrhalis* the switch to IgG3 production seemed to be age related, specific IgG3 being essentially undetectable in children under the age of 4 years (14). IgG1 and IgG3 are typically produced in response to protein antigens (18), with IgG1 present in significantly greater amounts than IgG3, but as yet, little is known about factors which may preferentially induce the production of IgG3. Although specific switch factors have been described for different IgG subclasses in the mouse, much less is known about this system in humans. Isotype switching to both IgG1 and IgG3 appears to be controlled by similar processes which may be regulated by the T-cell-derived cytokine interleukin 10 (3). Goldblatt et al. (14) suggest that the membrane-bound nature of the *Branhamella* proteins, and their mitogenic activity for B cells, may be partly responsible for the IgG3 antibody response. The propensity for MSP2 to induce IgG3 antibodies suggests that this antigen may be a useful tool for investigating subclass-specific switch mechanisms.

The most important question regarding the potential of MSP2 as a vaccine antigen is whether MSP2-specific immune responses are involved in protective immunity to malaria. We have shown here that MSP2 is naturally antigenic, that the immune response is directed to dimorphic as well as polymorphic regions of the molecule, and that these antibodies are of appropriate subclasses. Longitudinal epidemiological studies are now under way to determine whether these antibodies are

able to mediate immunity to clinical malaria and to investigate the hypothesis that polymorphism within the repeat sequences of MSP2 is a significant factor in the slow development of protective immunity to *P. falciparum.*

## ACKNOWLEDGMENTS

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