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Cellular and Humoral Immune Responses to Well-Defined Blood Stage Antigens (Major Merozoite Surface Antigen) of *Plasmodium falciparum* in Adults from an Indian Zone Where Malaria Is Endemic

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Conserved and variant regions of two blood stage vaccine candidate antigens of *Plasmodium falciparum*, merozoite surface antigen (MSA-1) and ring-infected erythrocyte surface antigen (Pf155/RESA), have been shown to be immunogenic. However, the relative immunogenicity of these immunogens in different populations has not been studied. The conserved N-terminal region of MSA-1 was investigated for its immunogenicity by studying cellular (T cell) and humoral (B cell) immune responses in *P. falciparum*-primed individuals, living in malaria-hyperendemic areas (Orissa State, India), where malaria presents an alarming situation. MSA-1-derived synthetic peptides contained sequences that activated T cells to proliferate and release gamma interferon in vitro. There was considerable variation in the responses to different peptides. However, the highest responses (51% [18 of 35] by proliferation and 34% [12 of 35] by gamma interferon release) were obtained with a synthetic hybrid peptide containing sequences from conserved N- and C-terminal repeat regions of MSA-1 and Pf155/RESA, respectively. Antibody reactivities in an enzyme immunoassay of plasma samples from these donors to different peptides used for T-cell activation were heterogeneous. In general, there was poor correlation between DNA synthesis and either gamma interferon release or antibody responses in individual donors, underlining the importance of examining several parameters of T-cell activation to assess the total T-cell responsiveness of a study population to a given antigen. However, the results from our studies suggest that synthetic constructs containing sequences from the N- and C-terminal regions of MSA-1 and Pf155/RESA representing different erythrocytic stages of the *P. falciparum* parasite are more immunogenic in humans living in malaria-hyperendemic areas of India who have been primed by natural infection.

Both antibody-dependent and antibody-independent effector mechanisms are involved in protective immunity to malaria. Although antibodies have been shown to be protective in malaria (38), the ability of an immunogen to induce effective protection depends to a great extent on the T-cell recognition sites (7, 10, 15). As the malaria vaccines presently under consideration are subunit vaccines based on isolated parasite antigens or fragments, it is of utmost importance to identify the immunologically active structures (epitopes) in conserved antigens. Furthermore, to circumvent possible genetic restrictions of the host immune response (31), to combat vaccine-induced antigenic variation in the parasite (31), and to increase immunogenicity (28), the vaccine will probably be made polyvalent by the inclusion of proteins containing epitopes from different infectious stages of the parasite (28). Moreover, the relative immunogenicities of these epitopes have to be tested for their ability to induce optimal immune responses in different populations who have acquired immunity through repeated malaria infections.

Candidate antigens for inclusion in a vaccine against the asexual blood stage of the malaria parasite *Plasmodium falciparum* are MSA-1 (major merozoite surface antigen) (19, 20) and Pf155/RESA (ring-infected erythrocyte surface antigen), an antigen which the parasite deposits in the erythrocyte membrane during merozoite invasion (30). MSA-1 has been shown to be highly immunogenic (5, 6, 13, 24, 27, 35). Immunization experiments performed with native protein, recombinant MSA-1, or peptides in monkeys (29, 40) as well as in humans (28) indicated that this antigen constitutes one of the potential targets of a vaccine against the blood stages of the malaria parasite. Similarities in the organization of the gene for this protein among other plasmodia have been reported (34). The results of passive immunization of *Aotus* monkeys (3) and recent epidemiological studies in humans in West Africa also suggest that antibodies to certain invariant (30, 31) Pf155/RESA epitopes may be protective (4). There are several reports of T-cell sites in MSA-1 and RESA in an African population (9, 23, 41, 45). However, there is a lack of information about T- and B-cell recognition sites in the invariant regions of MSA-1 and RESA in Indian areas where malaria is endemic.

More than two million cases of malaria are reported each year in India (39, 47). Nearly 35% of the infections are caused by *P. falciparum*. The current status of malaria indicates that almost one-third of the cases of *P. falciparum* infection and one-half of all malaria deaths in the country as a whole occur in the state of Orissa (49). In a recent cross-sectional study in Sundargarh region, Orissa State, slide positivity rates of up to 38% were found. Among children aged 2 to 9 years, 37.3% had an enlarged spleen (48). It is of great relevance and importance to identify the antigens relevant to the development of host-protective immunity in people living in malaria-endemic areas

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TABLE 1. Synthetic peptides

Peptide	Residues[a]	Sequence
P1[b]	260–279, 43–53	CLDNIKDNVGKMEDYIKKNKKPYSLFQKEKMVLG
P2[c]	260–279, 43–53 (925–940[d])	CLDNIKDNVGKMEDYIKKNKKPYSLFQKEKMVLGPGEENVEHDAEENVEEN
P3	197–212	KYLIDGYEEINELLYK
P4	279–291	KTIENINELIEES
P5	379–393	KIEEHEKEIKEIAKT
P6	456–469	ALNELNSFGDLINP
P7	521–535	LNDITKEYEKLLNEI

[a] Positions are from Tanabe et al. (42) except as noted. For P1 and P2, the N-terminal MSA-1 residues are listed first, followed by the C-terminal RESA residues.
[b] C was used at the N terminus to facilitate binding of the new peptide.
[c] G-P-G or P was used as a spacer to separate the different epitopes.
[d] Positions according to Favaloro et al. (11).

of India; identification of these antigens is essential for the development of alternative strategies for controlling malaria in India.

In this study, we investigated the T- and B-cell recognition domains of the defined blood stage vaccine candidates MSA-1 and Pf155/RESA. For this purpose, we synthesized peptides representing conserved N-terminal regions of MSA-1 and a hybrid peptide comprising sequences from the N-terminal region of MSA-1 and from the C-terminal repeat regions of Pf155/RESA. The abilities of these peptides to induce in vitro cellular responses were studied by T-cell proliferation and gamma interferon (IFN-γ) production in *P. falciparum*-primed donors from malaria-endemic areas of Orissa State, India. To look for humoral (B cell) responses against these peptides, plasma samples from individual donors were tested for the presence of antibodies by an enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Study subjects. Venous blood samples were obtained with informed consent from 35 adults (aged 18 to 50 years) living in malaria-hyperendemic areas of Orissa State, India, where malaria transmission is high (48, 49) and perennial. None of the donors had clinical malaria at the time of sampling, and their blood smears were negative for malaria parasites. Blood samples from 10 healthy adults (laboratory staff) with no history of clinical malaria were included as controls.

Preparation and fractionation of peripheral blood lymphocytes. Venous blood (between 15 and 20 ml) was drawn into heparinized tubes. Mononuclear cells were isolated by gelatin sedimentation and Ficoll-Hypaque (Histopaque 1119; Sigma) centrifugation (32). T cells were separated by rosette formation with neuraminidase-treated sheep erythrocytes. E-rosette-forming cells were separated from non-E-rosetting cells by Ficoll-Hypaque centrifugation (22). Adherent cells were obtained from peripheral blood mononuclear cells by incubation in plastic petri dishes in 50% heat-inactivated human AB^+ serum for 1 to 2 h at 37°C. Nonadherent cells were washed off, and adherent cells were recovered after overnight incubation at 4°C (43).

Proliferation assay. The procedure for the T-lymphocyte proliferation assay has been described in detail elsewhere (23). In brief, 5,000 adherent cells in complete tissue culture medium (HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid]-buffered RPMI 1640 medium supplemented with 2 mM L-glutamine, 25 μg of gentamicin per ml, and 10% human AB^+ serum) were cultured overnight with 0.2 ml of antigens (5 μg of parasite antigen, 5 μg of noninfected erythrocyte ghost antigen, and 1 μg of the peptides per ml) in triplicate wells of round-bottomed microtiter plates. After antigen pulsing, supernatants were removed, and 100,000 autologous T cells suspended in tissue culture medium were incubated for 5 days at 37°C. Then, 100 μl of cell-free culture supernatant was collected from each well for IFN-γ determination, and proliferation was assayed by determining incorporation of [^{3}H]thymidine (1 μCi per well) in 100 μl of complete tissue culture medium for 18 h. The results were expressed as a stimulation index (SI), defined as mean counts per minute (cpm) for the test cultures divided by the mean cpm for control cultures, as described previously (23). The mean for control unstimulated cultures from all donors was 713 ± 91 cpm (mean ± standard error of the mean [SEM]; 95% confidence limits, 623 to 903 cpm). On this basis, an SI of ≥2.5 was considered a positive result.

Antigen and peptide preparations. Merozoite extracts (Mz) from in vitro cultures of *P. falciparum* FBJ (India) and supernatants from a sonicate of normal erythrocytes (erythrocyte ghosts) were used as parasite and control antigens, respectively (30).

Peptides 13 to 52 amino acids long (Table 1) were used in this study. Peptides P1 and P3 to P7 represent residues from the conserved N-terminal region of the K-1 strain of *P. falciparum* (42). The P2 peptide includes the residues of P1 and a 16-amino-acid sequence (EENVEHDAEENVEENV) from the C-terminal repeat region of Pf155/RESA (11). All of the peptides were synthesized by a stepwise solid-phase procedure with the model 430A peptide synthesizer (Applied Biosystems, Foster City, Calif.) (24). Syntheses were carried out on a 0.5-mmol scale with phenylacetamidomethyl resin. The couplings were carried out with preformed test butyloxycarbonylamino acid symmetric anhydrides except for glutamine and asparagine, which were coupled as hydroxybenzotriazole esters. The following side chain-protecting groups were used: aspartic acid (*O*-benzyl), glutamic acid (*O*-benzyl), serine (benzyl), threonine (benzyl), tyrosine (bromobenzyloxycarbonyl), lysine (chlorobenzoyloxycarbonyl), and histidine (tosyl). Peptides were deprotected and cleaved from the resin with trifluoromethanesulfonic acid-trifluoroacetic acid-thioanisole and then purified by gel filtration on Sephadex G-25 (Pharmacia LKB Biotechnology Inc., Piscataway, N.J.) followed by high-pressure liquid chromatography (HPLC) on a C-18 column (Waters, Millford, Mass.). Elution was performed by the following protocol: solvent A, 0.1% trifluoroacetic acid in water; solvent B, 0.1% trifluoroacetic acid in 60% acetonitrile; linear gradient from 0 to 100% solvent B over 35 min at a flow rate of 1.5 ml/min; and detection at 226 nm.

The purity of the peptides was assessed by analytic reverse-phase HPLC, and further purification was carried out if necessary. The amino acid compositions of these synthetic

peptides were confirmed by amino acid analysis (24). All peptides were tested for cytotoxicity in cellular assays. None of the peptides was toxic. Peptides P3 to P7 were selected on the basis of high α-amphipathic scores (28.7, 20, 34.4, 50.9, and 46.4 for P3, P4, P5, P6, and P7, respectively) (The amphipathic scores of the peptides were predicted to include T-cell epitopes on the basis of analysis with the algorithm developed by Margalit et al. [26]), whereas peptides P1 and P2 were chosen as hybrid peptides to test the immunogenicities of these peptides. The residue cysteine was added to the N terminus of peptides P1 and P2 to facilitate coupling of new peptides. Amino acids G-P-G or P were used as spacers to separate the different epitopes.

Serology. A small aliquot of plasma was obtained from each blood sample and used for detection of *P. falciparum* antibodies by conventional immunofluorescence and for anti-Pf155/RESA antibodies by erythrocyte membrane immunofluorescence (gluteraldehyde-fixed monolayers of ring-infected erythrocytes) (30). Antipeptide antibodies were determined by using an ELISA as described before (31). Briefly, ELISA plates (Tarsson) were coated with the appropriate antigen. After being blocked with 1% bovine serum albumin, the plates were incubated with serial dilutions of the plasma overnight at 4°C. For screening of plasma samples, a dilution of 1:500 was found to be optimal and was subsequently used for all determinations of antibodies in whole plasma. Bound antibodies were detected by horseradish peroxidase-conjugated anti-human immunoglobulin with *o*-phenylenediamine as the substrate. The reaction was stopped with 8 N H_2SO_4, and the A_{490} was measured with a microplate reader. Samples were considered to be positive in antibody response if the optical density (OD) value was greater than the mean OD plus 2 standard deviations (SD) (>0.2) for the control samples.

IFN-γ determinations. For IFN-γ estimation, supernatants were collected after 5 days of incubation, when release was optimal. They were assayed undiluted in a two-site ELISA with the use of two monoclonal antibodies against human IFN-γ (kind gift from G. Andersson, Lund, Sweden) as described before (2). Human IFN-γ (Gg 23-901.530; 4,000 U per ampoule; National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.) was used as the standard. The limit of sensitivity was 2 U/ml, and supernatants containing less than this were considered negative (21, 45).

Statistics. Differences between the responses induced by malaria antigens and control antigen were analyzed by paired *t* tests. T- and B-cell responses were compared by linear regression analysis.

RESULTS

All 35 subjects were adults and permanent residents of a village in Orissa State, India, and thus were likely to have been exposed to similar levels of infection and also probably to similar strains of *P. falciparum* over many years. None of them had clinical malaria at the time of blood collection. All plasma samples were positive for *P. falciparum* antibodies by conventional immunofluorescence at a titer of ≥1:1,000 on air-dried infected erythrocytes, and 65% had antibodies to Pf155/RESA at titers ranging from 1:50 to 1:1,250 as determined by erythrocyte membrane immunofluorescence.

Induction of DNA synthesis (proliferation assay). To define potential T-cell-activating epitopes in MSA-1, the in vitro T-cell responses of *P. falciparum*-primed donors to synthetic peptides corresponding to the N-terminal region of MSA-1 and a hybrid peptide (MSA-1 plus RESA) were measured by induction of proliferation and by IFN-γ release. Five of seven

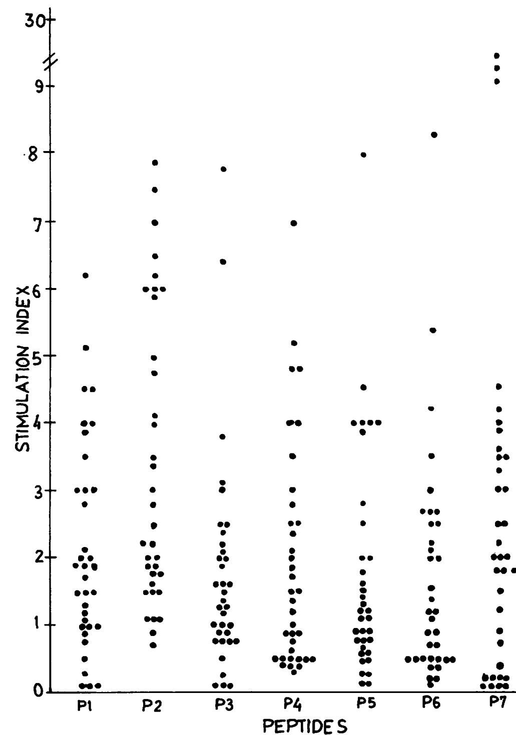

FIG. 1. Scatter diagram of DNA synthesis. DNA synthesis as determined by thymidine incorporation after 5 days of in vitro stimulation of purified T cells from 35 *P. falciparum*-primed donors with peptides derived from the N-terminal region of MSA-1 and MSA-1 plus RESA hybrid peptides was expressed as the SI.

peptides (Table 1) were selected on the basis of a high amphipathic score (see Materials and Methods).

Figure 1 shows the distribution of in vitro lymphocyte responses by proliferation to the seven peptides (Table 1) used in this study. All the peptides tested were able to induce cellular proliferation. There was considerable variation in the magnitude of individual responses to various peptides. Antigen-induced lymphocyte proliferation giving an SI of ≥2.5 was defined as a positive result in this study. By this criterion, the percentage of samples that gave a positive response to different antigens was determined (Table 2). Cells from the majority (74%) of primed donors proliferated in response to Mz, whereas a minority (14%) responded to the uninfected erythrocyte ghost (ghost) antigen. The response to Mz was significantly greater than the response to ghost antigen (paired *t* test, $P < 0.01$). Although all the peptides elicited cellular proliferation in 20 to 34% of samples (Table 2), the most frequent

TABLE 2. T-cell proliferation and IFN-γ responses[a]

Assay	% Positive samples								
	Mz	Ghost	P1	P2	P3	P4	P5	P6	P7
Proliferation	74	14	34	51	20	23	23	29	43
IFN-γ	43	17	34	34	23	29	26	20	20

[a] The percentage of T-cell responders to Mz and ghost antigen at 5 μg/ml and to peptides at 1 μg/ml is presented. Cells from *P. falciparum*-primed donors were tested for proliferation by thymidine incorporation and for IFN-γ release by ELISA after 5 days of culture with different antigens. The peptides used for these assays and their sequences are given in Table 1.

TABLE 3. Immune responses to three MSA-1-derived peptides in *P. falciparum*-primed adults[a]

Donor no.	Response								
	P1			P2			P7		
	SI	IFN-γ	Ab	SI	IFN-γ	Ab	SI	IFN-γ	Ab
r-23	−	+	+	+	+	+	−	+	+
r-8	−	+	+	−	+	+	+	−	+
r-6	+	−	+	+	−	+	−	−	+
r-1	+	−	+	+	−	+	+	−	−
r-15	+	−	−	+	−	−	−	−	−
r-19	+	−	+	+	−	+	+	−	+
r-30	+	−	−	+	−	−	+	−	−
r-20	+	−	−	+	−	−	+	−	−
r-21	−	−	−	−	−	−	+	−	+
r-16	−	−	−	−	−	−	−	−	−
r-17	−	−	−	−	−	−	−	−	−
r-25	−	+	+	+	+	+	−	+	+

[a] Stimulation of T cells from *P. falciparum*-primed donors with peptides P1, P2, and P7 was measured by thymidine incorporation and expressed as the SI. IFN-γ in stimulated T-cell culture supernatants was determined by ELISA. SI values of ≥2.5 and IFN-γ production of >2 U/ml were considered positive results (antigen specific). Peptide-specific antibodies (Ab) were assayed by ELISA. OD values of >0.2 were considered positive, as explained in the legend to Fig. 4. Symbols indicate a positive or negative response in each assay.

responses (51%) were obtained with the P2 peptide. However, approximately equivalent results (43%) were also obtained with a peptide (P7) derived from the N-terminal region of MSA-1 (Table 2). Moreover, a small fraction of the samples gave positive responses only with this peptide and not with the other peptides, indicating the probable existence of distinct epitopes in this sequence (Table 3).

Lymphocyte proliferative responses in control samples were low; the mean SI ± SEM for Mz or peptides was 1.5 ± 0.14 (range, 0.3 to 1.5). Proliferative responses to malarial antigens were significantly higher in samples from primed individuals than in control samples. The differences were statistically significant ($P < 0.01$ for P1 and $P < 0.05$ for P7). Similar baseline values were shown by others studying lymphocyte proliferation (36).

IFN-γ secretion in lymphocyte cultures. Supernatants from 5-day cultures were used for determination of secreted IFN-γ by ELISA. Figure 2 shows the IFN-γ responses of samples from malaria-primed donors to malaria antigens (Table 1). The magnitude of the response varied between donors. The majority of the responders had low responses in this assay, and there was no obvious difference between the peptides in this respect. An IFN-γ level of ≥2 U/ml was considered a positive response. The percentage of positive responders to each antigen in the ELISA is shown in Table 2. Altogether, 43% of the donors responded positively to Mz and 17% responded positively to the ghost antigen. Peptides P1 and P2 induced positive responses in 34%, and 20% of the donors responded to P7. The amount of IFN-γ in supernatants from stimulated cultures of control samples was below the limit of detection by ELISA. IFN-γ responses to malaria antigens were somewhat significantly higher in samples from primed individuals than in the controls ($P < 0.2$ for P2 and $P < 0.3$ for P1).

Serum antibody reactivities. Antibody binding to the seven peptides shown in Table 1 was determined for plasma samples by ELISA. Figure 3 shows the heterogeneous antibody reactivities of various donors to different sequences of MSA-1. Antibodies from most of the donors reacted to one or more peptides with different degrees of binding. In general, the

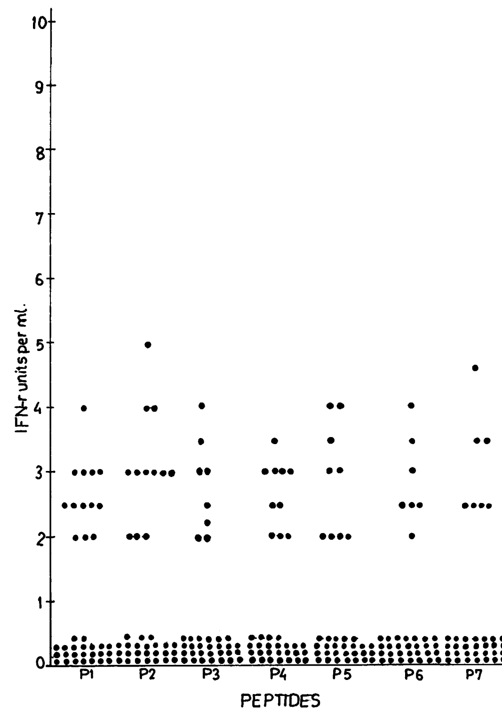

FIG. 2. IFN-γ production. Supernatants collected from T-cell cultures before thymidine pulsing were tested for IFN-γ release by ELISA.

concentrations of antibodies to various peptides were normally distributed. However, elevated responses at a higher frequency were found for peptides P2 and P7 (Fig. 3). Plasma samples with OD values greater than the mean OD plus 2 SD (>0.2) for 10 controls were considered positive. Analysis of the positive reactions in the peptide ELISA showed that P2, P7, and P1 reacted with 80, 77, and 69% of the samples, respectively (Fig. 4). The other five peptides, P3 through P6, were recognized less frequently (Fig. 4).

Heterogeneity of in vitro-induced immune responses; correlation analysis of T- and B-cell responses. Activation of T cells was measured as induction of IFN-γ secretion and/or T-cell proliferation. Correlations among T-cell responses (DNA synthesis and IFN-γ production) and between T- and B-cell responses (prevalence of antibody) to individual peptide were investigated. The results obtained with the three immunogenic peptides are given in Table 3. There was no correlation between these responses for individual donors (Table 3). While there was a significant negative correlation between proliferative response and IFN-γ release ($r = -0.02$ and $P < 0.1$ for P2), antibody and proliferative responses did not reveal any positive or negative correlation.

DISCUSSION

The blood stage vaccine candidates MSA-1 (19, 20) and Pf155/RESA (11, 30) have been reported to have T- and B-cell recognition domains (9, 23, 41, 43). In the present investigation, we studied the cellular and humoral (T- and B-cell) responses to synthetic peptides representing the conserved N-terminal region of MSA-1 (gp190) of *P. falciparum* K1 (42) in adults living in areas of India where malaria has been endemic for many years (48, 49). We also investigated whether a hybrid peptide (P2) comprising sequences representing the

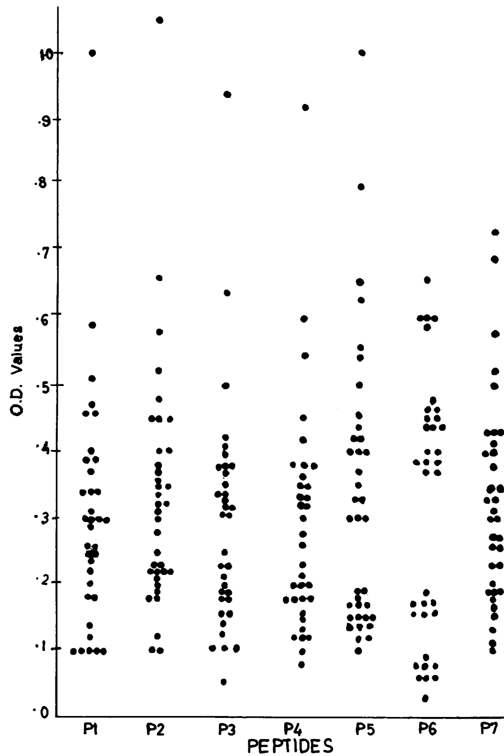

FIG. 3. Antibody profile. Binding of antibodies from 35 *P. falciparum*-primed donors to peptides representing different regions of the N terminus of MSA-1 and to hybrid peptides was determined by ELISA. The peptides used for these assays and their sequences are given in Table 1.

repeat sequence of the C-terminal region of Pf155/RESA (11) and MSA-1 (Table 1) can elicit improved T-cell responses.

To define potential T-cell-activating epitopes, the in vitro T-cell responses of samples from *P. falciparum*-primed donors to synthetic peptides (13 to 52 amino acids long) were measured by induction of proliferation and by IFN-γ release. T-cell responses to the peptides varied considerably. None of the peptides elicited a positive response in all the samples. In general, T cells from 30% of the donors did not respond to any of the peptides tested. This state of unresponsiveness has been attributed to immunosuppression (17, 18, 36), neonatal tolerance (33), and genetic restriction of immune responses (16). However, recent studies elsewhere (44) failed to detect associations between major histocompatibility complex (MHC) class II antigens and human immune response to Pf155/RESA antigens in *P. falciparum*-primed donors (44).

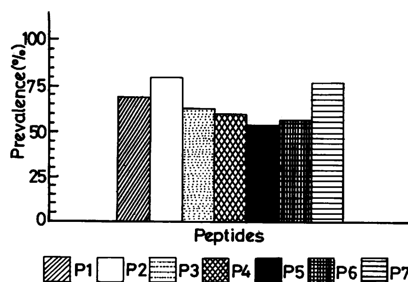

FIG. 4. The prevalence of antibodies to various sequences of MSA-1 (Table 1) was tested by ELISA. Plasma samples were considered positive in antibody response if the OD was greater than the mean OD ± 2 SD (>0.2) for the control samples.

While 74% responded positively to the merozoite lysate (Mz), 51 and 43% responded to peptides P2 and P7, respectively. The Mz antigen was relatively crude and certainly would be expected to contain more T-cell epitopes than the peptides (45). Some donors (14%) responded to the ghost antigen. Individuals exposed to malaria become sensitized to normal erythrocyte components (22). Of the peptides tested, P2 most frequently induced T-cell proliferative responses. Samples that responded to peptide P2 also responded to crude Mz lysate. The higher response seen with the P2 peptide than with the P1 peptide may suggest that additional T-cell epitopes in Pf155/RESA repeat sequences are being recognized by a fraction (18%) of Pf155/RESA-seropositive donors (data not shown). Moreover, the C-terminal repeat region of Pf155/RESA was shown to contain T-cell epitopes (23, 45). However, individual samples have to be tested with MSA-1- and Pf155/RESA-derived individual peptides to confirm this finding.

Many of the donors (43%) who responded significantly to P7 also responded to Mz lysate. T cells from 5 of 35 primed donors responded positively in the proliferation assay only to P7, indicating that this sequence may contain a distinct or cross-reactive epitope(s) recognized by the T cells from a fraction of the donors. Cross-reactions at the cellular level between peptides or small recombinant proteins have also been seen by others (37). However, more subjects need to be tested to investigate this question.

The reason for the few positive IFN-γ responses to MSA-1-derived peptides among primed donors (34%) may be the detection system (ELISA) used for estimation of positive responses. Low frequencies of IFN-γ responses were seen with MSA-1 recombinant proteins (35). Positive IFN-γ responses to peptides could probably be elicited at higher frequencies if sensitive IFN-γ detection methods are used (21).

Antigen-specific T-cell proliferative responses were shown to be higher in malaria-exposed samples than in the controls (35–37). In recent studies elsewhere, lymphocytes from nonexposed healthy individuals responded to synthetic peptides (12) at a high frequency. Relatively high concentrations of peptides (5 to 30 μg/ml) were used to get optimal responses in these studies, reflecting the possibility that saturation of the antigen may activate virgin cells (12). It is likely from our studies that the low T-cell responses seen in the controls may be due to the low antigen concentrations (1 μg/ml) used for stimulation, which were probably too low to activate the antigen-specific cells present at a low frequency in control samples.

The MSA-1-derived peptides tested in our studies occur in the recombinant protein termed 190.L. (14, 42), which contained T epitopes (35). Our study also defined some T-cell epitopes within the MSA-1 region. It is of interest to investigate for B-cell epitopes by studying the antibody reactivity in an ELISA against MSA-1-derived peptides. Most of the plasma samples showed positive reactivity against at least one peptide. Increased levels of antibodies against MSA-1 recombinant proteins were seen among acutely infected individuals (13); these antibodies were short lived in infants and children living in malaria-endemic areas with seasonal transmission (13), whereas antibody responses remained constant over the years in individuals living in malaria-endemic areas with perennial transmission (4). The antibody reactivity to all peptides seen herein may reflect the continuous boosting of individuals by natural infection under perennial transmission. In ongoing studies, the significance of these antibodies in protection is

being investigated. However, it will be difficult to know whether the antibodies against these peptides are due to multiple cross-reactivity to other malarial antigens. Cross-reactivities to plasmodial antigen in serum have been documented by others (1).

Most of the plasma samples reacted against the peptides P2 (80%) and P7 (77%), and 69% reacted against P1. Despite its short amino acid sequence (15 amino acids long), P7 was recognized by antibodies in 77% of the plasma samples and induced proliferative T-cell responses in 43%, suggesting that this peptide is highly immunogenic, containing distinct or overlapping T- and B-cell epitopes. Immune responses to peptides of short amino acid sequences from Pf155/RESA and clustered-asparagine-rich protein comprising both T- and B-cell epitopes were shown to occur at a higher frequency among primed donors (31, 46).

To assess the biological significance of antigen-specific T-cell responses (25), correlations among T-cell responses and between B- and T-cell responses (antibodies and DNA synthesis or IFN-γ release) for individual peptides were investigated. Both proliferation and IFN-γ production were negatively correlated with the concentration of corresponding antipeptide antibodies used for T-cell activation, which may indicate that proliferation assays do not measure the activation of T-helper cells which help the B cells. Proliferating and IFN-γ-producing cells may belong to functionally heterogeneous T-cell subsets (25), reinforcing the importance of using several parameters for T-cell activation when estimating the responses induced by different epitopes in a given population.

Concluding remarks. A number of studies on the B- and T-cell recognition domains of the blood stage vaccine candidates MSA-1 and Pf155/RESA have been published (9, 23, 45). Conserved and variant regions of these molecules have been shown to be immunogenic; however, the relative immunogenicity of these regions of both proteins in different populations has not been studied. In this study, we analyzed cellular and humoral responses against synthetic peptides representing the N-terminal region of MSA-1. The present results indicate that the N-terminal region of MSA-1 contains B- and T-cell epitopes which are recognized by the immune systems of individuals living in areas of India where malaria is a serious hazard (48, 49). The proliferative T-cell responses seen at higher frequencies with the MSA-1 plus RESA hybrid peptide in our studies emphasize the importance of combining different promising vaccine candidates to improve the efficacy of vaccines (28). Since the immunodominant T-cell domains from the N-terminal and C-terminal regions of MSA-1 and RESA have been shown to be conserved in different *P. falciparum* strains (14, 30), sequences recognized by T and B cells appear to be suitable components for inclusion in a subunit malaria vaccine. This study also underlines the importance of examining several parameters of T-cell activation for estimating the total proportion of individuals responding to a given epitope of any antigen.

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