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A Prospective Study of the Association between the Human Humoral Immune Response to *Plasmodium falciparum* Blood Stage Antigen gp190 and Control of Malarial Infections

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The human humoral immune response to the *Plasmodium falciparum* merozoite surface antigen gp190 was analyzed to determine the rate of reinfection by the parasite and the ability to control parasite density. The prospective study was carried out in a West African village where malaria is hyperendemic. No correlation between the antibody titers and protection against infection was observed within the group of children. Positive and negative associations of antibody specificities with protection against and/or control of parasitemia were, however, found for adolescents and adults, respectively. Thus, in adolescents, the presence of antibodies to gp190 fragment M6 correlates with a 50% reduced risk of *P. falciparum* infection and an increased ability to control parasitemia, whereas in adults, the humoral response to some of the polymorphic regions of gp190 associates with an increased risk of infection.

Repeated *Plasmodium falciparum* infections generally lead to the development of a nonsterile immunity in adults living in malaria-endemic areas. Although the mechanisms underlying this immunity are not well understood, the importance of the humoral immune response in the defense against *P. falciparum* infections was clearly demonstrated three decades ago by the groups of I. McGregor and S. Cohen (6), who showed that the transfer of gamma globulins from immune adults to infected children rapidly decreased parasitemia. These results were recently corroborated by an analogous, well-controlled study by Bouharoun-Tayoun et al. (2), who described the rapid and efficient decrease of parasitemia within 3 days when highly parasitized children were treated with purified gamma globulins from immune adults.

The identification of parasite antigens which contribute to this protective humoral response is therefore a most pertinent problem. By using parasite antigens produced in heterologous systems by recombinant DNA technology, several seroepidemiological studies have been carried out with the aim of correlating the presence of antibodies to defined antigens or portions thereof with protection against infection or against severe clinical symptoms. However, studies with the circumsporozoite protein and the ring-infected erythrocyte surface antigen of *P. falciparum* have so far yielded contradicting results (1, 10, 14, 24, 28, 29).

The major merozoite surface antigen (gp190) of *P. falciparum*, a blood stage antigen, is considered a promising vaccine candidate. Active immunization of *Saimiri* or *Aotus* monkeys with gp190 resulted in strain-specific complete or partial protection (7, 17, 26, 34, 35). In rodents, passive transfer of monoclonal antibodies directed to the respective gp190 analog of *P. yoelii* and *P. chabaudi* as well as active immunization with affinity-purified antigen were both able to protect mice from otherwise lethal infections with the respective parasite (16, 19, 23). Finally, gp190-specific antibodies were isolated from immune complexes formed when *P. falciparum* merozoites were cultivated in the presence of sera from immune individuals (21).

Sequence comparisons of the gp190 gene from several isolates revealed different degrees of homology in various parts of the molecule. This has served to divide the gp190 sequence into 17 structural blocks. Some of these blocks are highly conserved, whereas the majority are dimorphic. The gp190 genes of isolate K1 from Thailand and of MAD20 from Papua New Guinea (37) are prototypes for the two alleles causing this dimorphism. However, two small regions, blocks 2 and 4 (37), do not follow the dimorphic rule. For both blocks a third type of sequence has been discovered in the isolates RO33 and CSL2 (4, 27). Moreover, tripeptide repeats found in block 2 of several isolates are degenerate, resulting in a truly polymorphic region of the molecule.

In our laboratory we have subcloned sequences covering the entire dimorphic and conserved parts of K1 and MAD20 isolates as well as several sequences of blocks 2 and 4 and expressed them in *Escherichia coli*. Some of these recombinant fragments were recognized in immunoblot and enzyme-linked immunosorbent assay (ELISA) analysis by nearly 100% of individuals living in malaria-endemic areas (8, 25). Furthermore, we found that the antibodies detected by our approach were to a large extent isolate specific (8). When the antibody levels in such a population were monitored over a period with grossly alternating malaria indices, a clear correlation between malaria infections and gp190-specific antibody prevalence emerged. However, the dynamics of the immune response were different between adults and children; i.e., whereas children lost their gp190-specific antibodies rapidly after cessation of infection, the adult humoral response remained high even during the annual dry season with its low transmission rate.

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We were therefore interested in whether the presence of these antibodies would correlate with protection against reinfection in the following high-transmission season. We applied an experimental scheme used by Hoffman et al. (14) in a study of the protective value of circumsporozoite protein-specific antibodies.

MATERIALS AND METHODS

Study area and population. The study was carried out in the village of Safo, which is located 15 km northeast of Bamako, Mali, in the dry savanna. The climate is characterized by a rainy season from June to October, with maximum precipitation during August and September, and a dry season from November to May. The malaria transmission pattern reflects the seasonality of the rain falls. Safo is inhabited by about 1,000 individuals living either in clusters of houses or in compounds scattered around the village. The ethnic groups are Bambara (90%) and Malinke (6%), and there are a few Peul and Sarakolé individuals. Nearly all the villagers are farmers. Before this study, the village was visited for two serosurveys in 1988 and one in May 1989 (8).

Field methods. The study was approved by the National Ethical Committee of the Ministry of Health of Mali. During the study a physician from the Ecole Nationale de Médecine et de Pharmacie du Mali stayed in the village and was assisted by a local health worker. Free health care was provided throughout the study. Twenty-five families comprising 206 individuals were initially enrolled in the study after giving informed consent. On day 0 of the study (28 May 1989), 5 ml of venous blood was collected from each individual in Vacutainers (Becton Dickinson, Heidelberg, Germany). Children were bled from the dorsum of the hand with a 24-gauge needle. Capillary blood from finger pricks was used to prepare thick and thin smears. After the blood sampling, each individual was treated orally with a dose of 25 mg of chloroquine per kg of body weight on three different days. Chloroquine was chosen because a survey in March 1989, using the World Health Organization recommended in vivo test, had demonstrated the absence of resistance to this drug in Safo. Blood smears were made on days 7 and 14 and every 2 weeks thereafter. Monitoring was stopped 2 months after the onset of the dry season on day 208 (29 December 1989). Parasites detected in blood smears were not treated unless febrile episodes were noted.

Blood films and sera. Air-dried thick and thin blood films prepared in the field were stained with Giemsa stain (3% for 45 min). Parasitemia was evaluated by counting the infected erythrocytes in high-power fields containing 300 leukocytes. Parasite densities were calculated by assuming a leukocyte count of 7,500/μl. This leukocyte density had been established before specifically for this village as a mean of 200 blood samples obtained from adults and children for hemoglobin electrophoresis.

To obtain sera, we allowed blood samples to clot at room temperature. After centrifugation, sera were separated from the pellets and stored at −20°C for 2 weeks and at −80°C after transfer to Heidelberg.

Hemoglobin genotype. Blood samples were subjected to electrophoresis on acetate sheets, and the genotype of the hemoglobin was identified by its mobility as described by Willcox et al. (38).

gp190 antigen fragments. The gp190 antigen sequences (Table 1; Fig. 1), previously expressed in *Escherichia coli*, yielding fusions to chloramphenicol acetyltransferase or dihydrofolate reductase (8, 25) were subcloned into plasmid pDS781,6xHis (36) in such a way as to eliminate the nonmalarial fusion moiety as described by Früh et al. (8). The resulting proteins contained at their N terminus a stretch of 6 histidine residues which allows their efficient isolation by nickel chelate chromatography (13).

TABLE 1. gp190 fragments used for antibody detection

Parasite isolate	Fragment[a]	Amino acid position[b]	Homology[c]
K1	K2.1	56–108	P
	F2	106–321	C, P
	K4.1	311–340	P
	F4	469–714	D
	F5	671–833	D
	F7	915–1100	C, D
	F9	1194–1439	D
	F10	1412–1608	C, D
MAD20	M2.1	56–111	P
	M3	123–302	C
	M4.1	314–345	P
	M6	384–595	D
	M7	595–897	D
	M9	1078–1251	C, D
	M10	1250–1398	D
	M11	1397–1563	D
RO33	R2.1	56–105	P
	R4.1	308–337	P

[a] All of the fragments have been subcloned from constructs described previously (8, 25), with the exception of K2.1, M2.1, and R2.1, which were amplified from genomic DNA by polymerase chain reaction for cloning, and K4.1, M4.1, and R4.1, which were chemically synthesized.

[b] Amino acid positions are from references 4, 22, and 37 for the respective isolate (column 1).

[c] Classification of sequences into conserved (C), dimorphic (D), and polymorphic (P) follows the proposal of Tanabe et al. (37).

Sequences from the block 2 polymorphic region of isolates MAD20, K1, and RO33 (M2.1, K2.1, and R2.1) were amplified by the polymerase chain reaction, using standard reaction conditions (31) and 1 μg of genomic DNA prepared from in vitro cultures. The following primers specific for the flanking sequences were obtained by oligonucleotide synthesis: MAD20, 5′-ATTAGGATCCGGAACAAGTGGAACA GCTG-3′ and 3′-GGAAGTCTATTAAGTCCTAGGAGTT-5′, K1, 5′-CTTAGGATCCGAAGAAATTACTACAAAAG GTGC-3′ and 3′-CGGGAGGTCGACTACGTCCTAGGAG CT-5′; and RO33, 5′-ATTAGGATCCGGAGCAAATACT CAAGTTGTTGC-3′ and 3′-CGTTTAGGAAGTCTACTA AGTCCTAGGAGTC-5′. The priming sequences carried *Bam*HI restriction sites at their 5′ termini. DNA was amplified in a Techne PHC-2 thermocycler programmed for 30 cycles as follows: 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. Amplification was terminated with a 10-min incubation at 72°C. Polymerase chain reaction products were digested with *Bam*HI, purified by agarose gel electrophoresis, and cloned into pDS781,6xHis linearized with *Bam*HI and *Bgl*II. Clones were verified by sequencing by the dideoxy-chain termination method (32).

The sequences were expressed in *E. coli* SG13009 and purified by nickel chelate affinity chromatography as described previously (8). Purified gp190 fragments were stored at 4°C in 6 M urea. Peptide sequences spanning block 4 of MAD20, K1, and RO33 (M4.1, K4.1, and R4.1) were synthesized chemically by solid-phase techniques and purified by high-performance liquid chromatography.

ELISA. Antibody levels were assayed by transferable

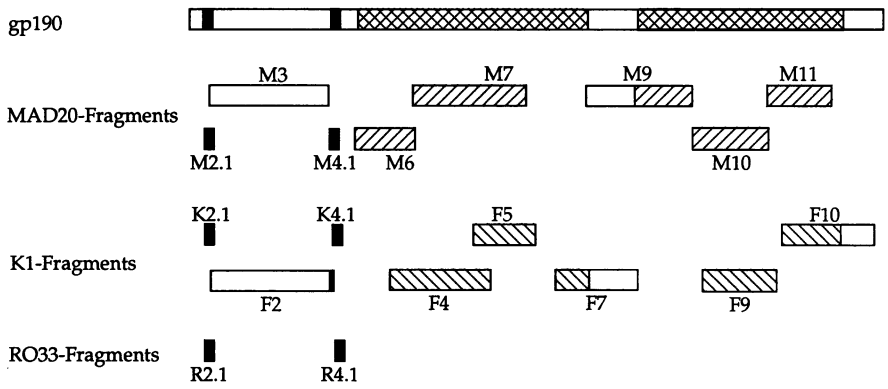

FIG. 1. Schematic representation of the gp190 allele and the fragments used in this study. Open boxes indicate conserved regions; hatched boxes indicate dimorphic regions; and black boxes indicate polymorphic regions.

solid-phase ELISA (Nunc, Wiesbaden, Germany), which is based on the FAST-ELISA system (11). gp190 fragments were dissolved in phosphate-buffered saline (PBS), and the pins of the lid were sensitized by immersion into 100 μl of antigen solution overnight. Optimal concentrations of antigen were identified as shown in Table 2.

The sensitized pins were successively transferred for overnight incubation onto new microtiter plates containing 200 μl of 1% bovine serum albumin (BSA) in PBS, for 2 h into 100 μl of human sera diluted 1:100 in PBS–1% BSA–1% Tween 20, and finally for 1 h into 100 μl of goat anti-human immunoglobulin G conjugated to alkaline phosphatase (Promega, Heidelberg, Germany) and diluted 1:7,500 in PBS–1% BSA. Before and after incubation with the second antibody, pins were rinsed with PBS–0.3% Tween 20, and before development they were also rinsed with deionized H_2O. Following a 30-min incubation in 100 μl of substrate solution (1 mg of *p*-nitrophenylphosphate per ml in diethanolamine buffer [pH 9.5] and 0.5 mM $MgCl_2$), the optical density at 405 nm (OD_{405}) was measured in a Titertek spectrophotometer for duplicate samples. Cutoff values for positive responses (Table 2) were calculated as the mean OD_{405} plus three standard deviations for 20 serum samples from European patients suffering from diseases unrelated to malaria (9). Interplate variation was controlled by high-response standard sera in three different dilutions on each plate.

TABLE 2. Parameters of the gp190 ELISA

Parasite isolate	Fragment	Concn[a] (μg/100 μl)	Median[b] (OD_{405})	Range[b] (OD_{405})	Cutoff[c] (OD_{405})
K1	K2.1	0.10	0.06	0–0.92	0.10
	F2	0.50	0.36	0.09–1.27	0.44
	K4.1	0.25	0.04	0–0.60	0.10
	F4	0.50	0.28	0.05–1.13	0.28
	F5	0.25	0.22	0.01–0.71	0.38
	F7	1.00	0.19	0.03–0.71	0.32
	F9	0.50	0.15	0.01–1.02	0.38
	F10	1.00	0.28	0.05–0.79	1.02
MAD20	M2.1	0.25	0.13	0–0.87	0.10
	M3	1.00	0.10	0.01–0.46	0.19
	M4.1	0.50	0.04	0–0.65	0.10
	M6	1.00	0.57	0.05–1.23	0.35
	M7	0.50	0.40	0.02–0.96	0.31
	M9	1.00	0.13	0.02–0.44	0.27
	M10	0.25	0.13	0.01–0.69	0.27
	M11	1.00	0.32	0.02–0.72	0.32
RO33	R2.1	0.10	0.11	0–0.71	0.13
	R4.1	0.50	0.04	0–0.35	0.10

[a] Optimal concentrations were determined by serial antigen dilutions with high-titer standard sera.
[b] Median and range are given for the sera of the study population (n = 187).
[c] Cutoff values were determined as the mean plus three standard deviations for the sera of 20 Europeans suffering from diseases unrelated to malaria (9).

Statistical methods. Differences in antibody levels were analyzed by the Wilcoxon rank sum test. Differences in frequencies were tested by χ^2 analysis. SAS 5.18 statistical software running under the operating system CMS was used for all analyses (33). Stepwise proportional-hazards linear-regression models using maximum-likelihood estimates were fit by using the PHGLM procedure made available in the SUGI supplemental library (12). Age groups were defined as follows: children, 1 to 5 years; adolescents, 6 to 14 years; adults, 15 years and older. The level of significance was set to $\alpha \leq 0.05$.

RESULTS

Malaria in the study population. Of the 206 persons enrolled, 15 had to be excluded from the study because of missing serum samples, lack of participation in the biweekly visits, or noncompliance with chloroquine treatment, which was identified by parasitemia in day 7 blood smears. Resistance to chloroquine was not observed. Four serum samples were too small for the ELISA.

The parasite prevalence in the population and its development during the course of the study are shown in Fig. 2A, and the respective geometrical mean of positive parasite densities is shown in Fig. 2B. There was a clear age dependency of the parasite prevalence. A steep increase in the incidence was observed in children during the transmission season, culminating in 100% parasite prevalence in the youngest children on day 208. The incidence of parasitemia in adults (15 years and older), however, increased only slightly compared with the initial values. Cumulative malaria cases and new infections during the study period are shown in Fig. 3. Throughout the study period 15 persons remained free of infections. Of these individuals 14 were adults (16% of the age group) and 1 was an adolescent aged 12 years (1.6% of the age group).

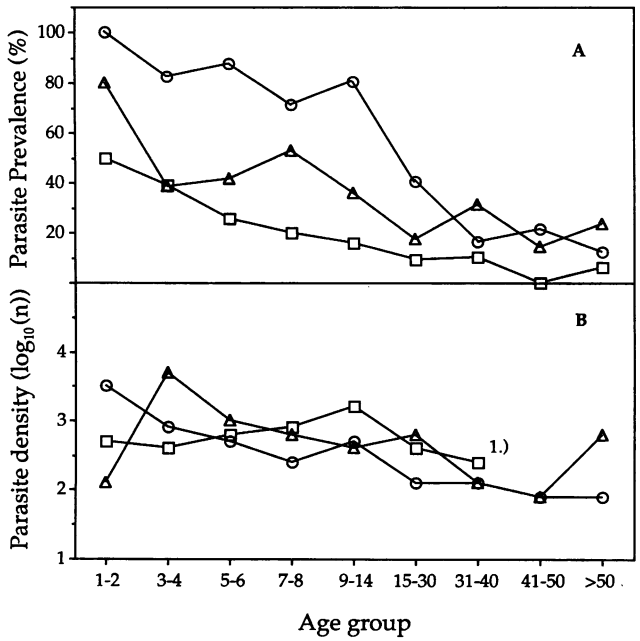

FIG. 2. Parasite prevalence and density by age groups on days 0, 84, and 208 of the study. (A) Parasite prevalence (percentage of *P. falciparum*-positive thick smears). (B) Positive parasite density [$\log_{10}(n)$] of the geometric mean of the number of *P. falciparum* asexual parasites per microliter of blood in subjects with a positive blood film]. 1.) On day 0, no infections were found in the 41- to 50-year age group and only one person older than 50 was infected. Symbols: □, day 0; △, day 84; ○, day 208.

Hemoglobin analysis. Samples for hemoglobin electrophoresis were obtained from 186 individuals. Of these individuals, 69.9% had hemoglobin AA and 14% showed the sickle cell trait (AS). None was homozygous for hemoglobin S. Hemoglobin C was heterozygously expressed in 13% of the persons, and homozygously expressed in 5%. Of 118 individuals who expressed hemoglobin AA, 22 (15.2%) were parasitized on day 0 whereas no parasites were found in the 26 sickle cell trait carriers. These frequencies were significantly different when tested by χ^2 analysis of a 2×2 table ($P = 0.017$).

Analysis of sera for gp190-specific antibodies. The prevalence of immune recognition of the gp190 fragments ranged from no positive reactions at all against F5 and M9 in children to almost 90% prevalence of antibodies against M6 in adults (Fig. 4A). M7, M2.1, and M11 were also recognized with high frequency. Of the K1-type fragments, F2 and F4 reacted most frequently. The dependence of seropositivity on age was observed for all the fragments. In general, the prevalence of positive reactions increased from childhood through adolescence to adulthood. These differences between age groups were even more pronounced when only nonparasitized individuals were compared (Fig. 4B). Responses to fragments M3, K2.1, F7, and F9, however, were similar in children and adolescents. Only with the onset of adulthood were higher prevalences detected.

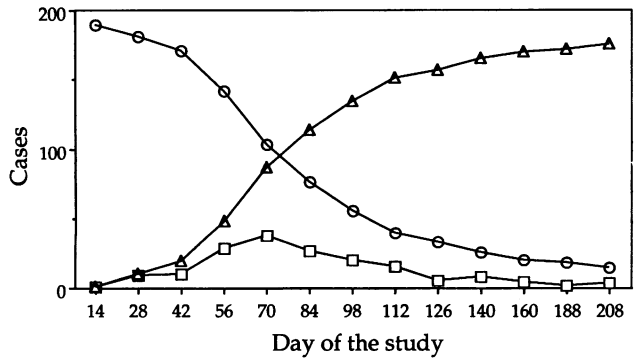

FIG. 3. *P. falciparum* infections during the time course of the study. The number of new cases (□), cumulative number of new cases (△), and number of subjects who remained free of parasites by the indicated day of study (○) are shown.

When analyzing F10, we found that normal sera reacted strongly. This made it difficult to distinguish specific from nonspecific binding (Table 2). The reactivity of the control sera with F10 is probably due to the presence of nine cysteine residues in the C terminus of this fragment, which might cause high structural variability and a concomitantly increased cross-reactivity.

Cross-sectional analysis of the data obtained on day 0. Children showed significant differences in their antibody responses to 13 of 18 gp190 fragments when grouped according to the presence or absence of parasitemia at the time of blood sampling (Table 3). In adolescents this was still true for eight fragments. In adults no significant differences were observed. Whenever significant differences in the humoral response between the infected and noninfected groups were detected, the higher antibody responses were always associated with the presence of parasites. Thus, in the cross-sectional analysis the antibody response was an indicator of a persistent infection rather than a sign of protection in children and some adolescents.

The median humoral responses to the gp190 fragments given in Table 3 showed a strong age dependency when noninfected children, adolescents, and adults were compared. The values in children were often less than half those in adults. Infected children, however, displayed a median OD_{405} which reached 80% or more of the level in noninfected or infected adults for fragments F7, F9, F10, M3, M4.1, M6, M7, and M9.

Predictive value of the antibodies. Proportional-hazards linear-regression models were generated by using gp190 ELISA data, age, sickle cell trait, and parasitemia on day 0 as independent startup variables. Because no positive reactions were detected for F10, these data were excluded from the models. Antibody levels to the gp190 fragments were dichotomized by using the cutoff value generated with the control sera. This approach was chosen because the relationship of antibody titers and failure time variables are not necessarily correlated in a linear manner. The data set was evaluated by using two models for the three age groups. In one model, failure time was defined as the day when patent parasitemia was detected for the first time. In the other model, failure time was defined as the day when the parasite density first reached 5,000 or more infected erythrocytes per μl. Whereas the first definition serves as a parameter for maintenance of sterile immunity, the second definition can be seen as an indicator of the ability to control parasitemia.

The resulting models are shown in Table 4. Factors predicting both protection (relative risk estimates, <1) or increased malaria risks (relative risk estimates, >1) were included in the model for the adolescents. Among the nonhumoral factors, older age and the sickle cell trait were associated with prevention of infection. Antibodies against

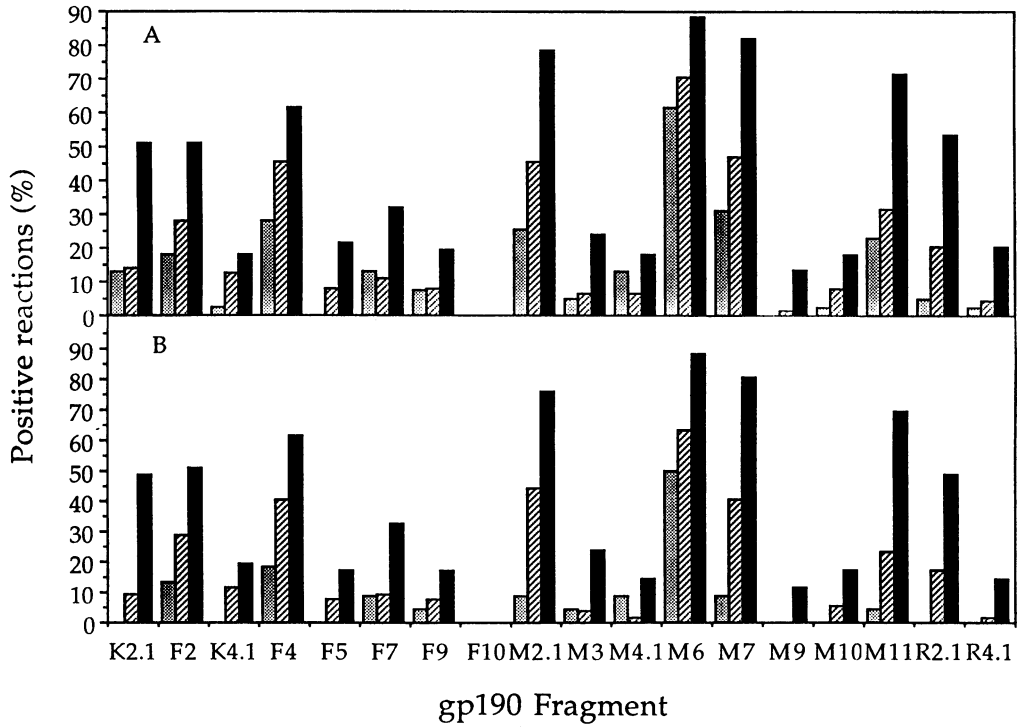

FIG. 4. Percentage of positive reactions to gp190 fragments as determined by ELISA for children, adolescents, and adults. Age groups were defined as follows: children, 1 to 5 years (▩); adolescents, 6 to 14 years (▨); adults, 15 years and older (■). (A) Prevalence in all individuals of the age groups. (B) Prevalence in nonparasitized individuals only.

fragment M6 (Fig. 4) reduced by half both the infection risk and the risk of insufficient control of parasitemia. The M11 response was associated with a 2.6-fold-higher risk of an early infection. No significant predictor variables were found in children no matter which failure time definition was used. Adults displayed antibody titers that were associated with a higher risk of developing *P. falciparum* malaria. Higher titers against the R2.1 and R4.1 sequences indicated a

TABLE 3. Comparison of humoral responses to gp190 fragments between noninfected and infected children, adolescents, and adults by the Wilcoxon rank sum test

Parasite isolate	Fragment	Children			Adolescents			Adults		
		Median OD_{405}		*P*	Median OD_{405}		*P*	Median OD_{405}		*P*
		Noninfected (n = 23)	Infected (n = 15)		Noninfected (n = 53)	Infected (n = 10)		Noninfected (n = 80)	Infected (n = 6)	
K1	K2.1	0.02	0.04	0.0056	0.04	0.07	NS[a]	0.11	0.12	NS
	F2	0.27	0.25	NS	0.32	0.34	NS	0.47	0.40	NS
	K4.1	0.03	0.04	0.0023	0.03	0.05	NS	0.06	0.05	NS
	F4	0.19	0.25	NS	0.24	0.31	NS	0.34	0.32	NS
	F5	0.18	0.19	NS	0.21	0.25	NS	0.25	0.30	NS
	F7	0.14	0.18	0.0074	0.18	0.20	NS	0.21	0.22	NS
	F9	0.05	0.22	0.0012	0.12	0.15	NS	0.19	0.23	NS
	F10	0.24	0.27	NS	0.24	0.32	NS	0.32	0.33	NS
MAD20	M2.1	0.04	0.09	0.0019	0.08	0.17	NS	0.23	0.21	NS
	M3	0.08	0.10	NS	0.08	0.11	NS	0.11	0.15	NS
	M4.1	0.02	0.05	0.0041	0.03	0.06	0.0016	0.05	0.03	NS
	M6	0.31	0.72	0.0003	0.47	0.64	0.0044	0.74	0.81	NS
	M7	0.11	0.45	0.0002	0.25	0.50	0.014	0.53	0.58	NS
	M9	0.08	0.13	0.0045	0.10	0.17	0.0062	0.15	0.21	NS
	M10	0.05	0.10	0.0050	0.09	0.15	0.020	0.16	0.24	NS
	M11	0.15	0.25	0.0006	0.23	0.38	0.0036	0.40	0.55	NS
RO33	R2.1	0.02	0.04	0.022	0.03	0.10	0.022	0.13	0.19	NS
	R4.1	0.01	0.03	0.0012	0.02	0.04	0.0049	0.06	0.07	NS

[a] NS, not statistically significant.

TABLE 4. Predictive value of antibodies to gp190 fragments and nonimmune factors for subsequent sterile immunity or control of parasitemia analyzed by proportional-hazards linear-regression models

Failure time definition[a]	Age group	Factor	Relative-risk estimate[b]	95% CI[c]	*P*
Sterile immunity	Adolescents	Age	0.77	0.63–0.93	0.008
		HbS	0.39	0.18–0.86	0.019
		M6[d]	0.46	0.23–0.94	0.032
		M11[d]	2.63	1.28–5.41	0.009
	Adults	R2.1[d]	2.37	1.40–3.99	0.001
		R4.1[d]	1.95	1.00–3.82	0.050
Controlled parasitemia	Adolescents	M6[d]	0.44	0.21–0.92	0.030
		M9[d]	13.5	1.57–115	0.020
	Adults	M4.1[d]	5.04	1.30–18.9	0.020

[a] Failure times were defined as the day of the first detection of parasitemia (sterile immunity) or the day when the concentration of 5,000 parasites per μl was first exceeded (control of parasitemia).
[b] Values of relative-risk estimates of <1 indicate protection; values of >1 relate to increased susceptibility.
[c] 95% confidence interval (CI) of relative-risk estimate.
[d] OD_{405} dichotomized at cutoff values (mean plus three standard deviations) of control sera.

decreased ability to maintain sterile immunity. The immune response against the M4.1 fragment is associated with a fivefold-increased risk of developing poorly controlled malaria infections.

DISCUSSION

The analysis of infection risk and antibody response to individual fragments of gp190 revealed a significant positive correlation: in the group of adolescents the humoral response against fragment M6 of the MAD20 isolate correlates with both a 50% reduction of the risk of infection and a 50% reduction of the risk of developing high parasite densities (Table 4). Antibodies recognizing M6 are most prevalent in the population studied and reach, on average, the highest titers observed in the group of adults (Fig. 4; Table 3). Moreover, they are indicative of the infecting parasite strain (8). Fragment M6 is located within the 83-kDa processing product of gp190, which is found at the surface of merozoites as well as in the supernatant of merozoite cultures (15, 18). It is identical to block 6 of the scheme proposed by Tanabe et al. (37), but lacks 3 amino acids at the N terminus and 15 amino acids at the C terminus. The dimorphism of the gp190 molecule is very pronounced in this region: on one hand, the MAD20 and K1 sequences show only a low degree of homology, whereas on the other hand, there is high conservation between the two alleles. There are only three positions known where more than two different amino acids have been found. The small amount of variability in this region is thus a promising prerequisite for immunizations.

The data obtained in this study do not allow conclusions about the role of the respective K1 type region of gp190 because K1-type parasites were either absent or had a low abundance at the time of the study (8). Thus, it would be necessary to carry out a similar analysis in a region where K1-type parasites or mixed genotypes are common. Interestingly, M6 has a 52-amino-acid overlap with a fragment of the CAMP strain which is recognized by antibodies that inhibit merozoite dispersal (20).

Antibody specificities which correlate positively with an increased risk of infection and/or a decreased ability to control parasitemia were observed in adolescents and adults. Although two of these specificities recognize conserved and dimorphic portions of the C-terminal part of gp190, three are directed toward polymorphic regions, namely R2.1, R4.1, and M4.1. These results resemble those obtained with ring-infected erythrocyte surface antigen peptides (1) and are reminiscent of reports which show that naturally acquired human immunoglobulins can promote parasite growth in vitro (2, 3). Such data may suggest a disease-promoting role for antibodies directed toward specific regions. On the other hand, the positive correlations between antibodies directed toward polymorphic regions of gp190 more probably reflect a history of numerous infections and therefore may reveal individuals with high susceptibility to infection.

When the antibody responses to gp190 on day 0, i.e., before the onset of the 1989 transmission season, are subjected to statistical testing, the following picture emerges. In children, antibody titers are extremely low unless the individuals are parasitized. Thus, the presence of parasites correlates with a significantly higher immune response. This also holds, although to a lesser extent, for adolescents. By contrast, adults maintain high antibody titers whether or not they are parasitized. Thus, when noninfected individuals are compared at the end of the dry season, the antibody response increases significantly with age. This supports the earlier observation of Früh et al. (8) that the antibody response to gp190 in children is short lived. Rooth et al. (29) have reported a decrease in the response to blood stage peptides within only 28 days after the chemotherapeutic clearance of parasites. Interestingly, in parasitized children, the antibody response is boosted and the difference in titers when compared with those in adults is abolished for a number of fragments (Table 3). Proportional-hazards analysis shows, however, that the increased antibody response due to parasitemia on day 0 has no effect on subsequent infections or parasite densities. Thus, the view that a persisting low parasitemia in children during the dry season might provide a certain degree of protection against the infection pressure during the rainy season, as a result of the preceding immune stimulation, is not supported by our data. Similarly, our data do not confirm the observation of Chizzolini et al. (5), who describe a correlation between high antibody titers against the 31.1 fragment of gp190 and absence of parasitemia.

The distinct pattern of seropositivity found by using the ELISA technique is in accordance with the data of Früh et al. (8) generated by analyzing immunoblots. By using the ELISA technique, a higher degree of seropositivity is observed; this is most probably due to a better discrimination of positive reactions by an experimentally defined cutoff than by the cautious visual judgment of immunoblots. As

observed previously (8), MAD20-specific sequences were recognized most often because of the prevalence of this allele in the parasite population at that time. Within the group of K1-specific fragments, two minor discrepancies with the previous immunoblot data were identified: (i) F2 was recognized more often than F5, which could be due to differences in the epitopes made available by these two techniques; and (ii) the highest percentage of positive reactions among the K1 series of fragments was obtained for F4, which had not been analyzed previously.

The results of the study presented here must be interpreted with caution for a variety of reasons, especially the following. (i) The use of a set of antigenic fragments certainly permits a more differentiated analysis of the humoral immune response. However, in principle this approach requires multiple comparisons and consequently an appropriate adjustment of the significance level. Here we have deliberately abstained from such adjustments to avoid a mistakenly rejected null hypothesis which would increase the possibility of overlooking meaningful associations (30). (ii) The gp190 fragments used do not cover the entire molecule, and most probably not all epitopes present in the native molecule are mimicked by the recombinant protein. On the other hand, the fragments could display determinants which are hidden under native conditions. (iii) Malaria was monitored as peripheral-blood parasitemia, which does not necessarily reflect the clinical status.

Nevertheless, we believe that the associations between anti-gp190 antibodies and malarial infections uncovered in this field study, which at the same time confirms the well known associations of sickle cell trait and age with the disease, will stimulate further analysis of the protective potential of gp190 as an immunogen and should be considered in the design of an antimalarial vaccine.

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