Acquisition of Invasion-Inhibitory Antibodies Specific for the 19-kDa Fragment of Merozoite Surface Protein 1 in a Transmigrant Population Requires Multiple Infections

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Antibodies against the 19 kDa C-terminal fragment of merozoite surface protein 1 ($MSP1_{19}$) are a major component of the invasion-inhibitory response in individuals immune to malaria. We report here the acquisition of $MSP1_{19}$-specific invasion-inhibitory antibodies in a group of transmigrants who experienced their sequential malaria infections during settlement in an area of Indonesia where malaria is highly endemic. We used 2 transgenic *Plasmodium falciparum* parasite lines that expressed either endogenous $MSP1_{19}$ or the homologous region from *P. chabaudi* to measure the $MSP1_{19}$-specific invasion-inhibitory antibodies. The results revealed that the acquisition of $MSP1_{19}$-specific invasion-inhibitory antibodies required 2 or more *P. falciparum* infections. In contrast, enzyme-linked immunosorbent assays on the same serum samples showed that $MSP1_{19}$-specific antibodies are present after the first malaria infection. This delay in the acquisition of functional antibodies by residents of areas where malaria is endemic is consistent with the observation that multiple malaria infections are required before clinical immunity is acquired.

Malaria remains a serious public health problem and a major cause of economic disadvantage in tropical and subtropical countries. The increasing incidence of antimalarial drug resistance and insecticide resistance has made more urgent the need to discover alternative control measures, including subunit vaccines composed of various parasite proteins. Proteins expressed on the merozoite surface are considered prime candidates for malaria vaccines because of their accessibility for antibody binding, which could lead to inhibition of parasite multiplication by interfering with the invasion of red blood cells. One such vaccine candidate that has been extensively studied is the conserved 19 kDa C-terminal fragment of merozoite surface protein 1 ($MSP1_{19}$). $MSP1_{19}$ has been used widely in many types of immunization protocols in model systems of malaria infection and shows a repeated ability to induce host-protective immune responses. Several immunoepidemiological studies have been conducted by using standard ELISA to measure the anti-$MSP1_{19}$ Ig titers in serum samples from individuals living in malaria-endemic areas. Even though most of the serum samples recognized $MSP1_{19}$ [1], the correlation between the presence of $MSP1_{19}$-specific antibodies and the clinically defined state of immunity in the study population was found to be inconsistent [2–8].

In an attempt to relate the observed state of immunity to specific antiparasitic immune responses, most efforts have focused on detecting correlations with antibody levels because of the observation that immunity to

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blood-stage parasites in humans is predominantly antibody based. Attempts have also been made to determine whether anti-$MSP1_{19}$ antibodies are capable of inhibiting parasite growth in vitro. A technical difficulty with such studies is that different types of antisera, including monoclonal antibodies, can have nonspecific inhibitory actions; therefore, the growth inhibition observed may be the result of factors such as the presence of antimalarial drugs ingested or additives to the serum, rather than specific antibodies [9].

Recently, O'Donnell et al. have solved this problem by developing 2 isogenic *Plasmodium falciparum* transfectant lines, which differ only in their $MSP1_{19}$ region, to examine the specific inhibitory activity of $MSP1_{19}$ antibodies in human serum samples [10]. By calculating the difference between the inhibition of invasion obtained when test serum samples were added to cultures of D10-*Pf*M3' (*P. falciparum* D10 with its homologous $MSP1_{19}$ region) with that obtained with D10-*Pc*MEGF (an isogenic *P. falciparum* line with its $MSP1_{19}$ region replaced with that of the rodent malaria species *P. chabaudi*), it was possible to determine the relative contribution of *P. falciparum* $MSP1_{19}$ (*Pf*$MSP1_{19}$)–specific antibodies to the overall invasion-inhibitory response. The results demonstrated that $MSP1_{19}$-specific antibodies in the serum samples from individuals living in Papua New Guinea made a significant contribution to the inhibition of *P. falciparum* invasion. A similar result was found in another study that used serum samples from semi-immune individuals in Western Kenya, Africa, and suggested that the measurement of $MSP1_{19}$-specific invasion-inhibitory antibodies may serve as a more accurate correlate of protection than total Ig measurement in assessing the protective efficacy of $MSP1_{19}$ during vaccine trials [11].

In the above-mentioned studies, the sets of serum samples were collected from individuals who had lived in areas of intense malaria transmission for their entire lives. Hence, it was not possible to answer the question of how rapidly nonimmune individuals can acquire an anti-$MSP1_{19}$ inhibitory immune response. Dent et al. have examined development of anti-$MSP1_{19}$ responses in infants born to mothers who had been exposed to malaria and concluded that maternal exposure had a major effect on responsiveness in 6–24 month -old children [12]. In the present study, we examined serum samples collected from Javanese transmigrants (nonimmune individuals) who developed their first malaria infections during an 18-month period of settlement in Armopa (Papua), a highly malaria-endemic area. Using these serum samples, we were able to follow the kinetics of acquisition of the specific invasion-inhibitory response to $MSP1_{19}$.

MATERIALS AND METHODS

Study design and subjects. The subjects and study design have been described elsewhere [13, 14]. In brief, a cohort of Javanese transmigrants was followed up for malaria infection in a new settlement located at Armopa in northwestern Papua (formerly Irian Jaya), Indonesia. Perennial transmission of malaria, primarily *P. falciparum* and *P. vivax,* occurred in that area, with an incidence of 1.5 *P. falciparum* infections and 1.8 *P. vivax* infections per subject in the first year of this study. During their first settlement, participants (age range, 6–58 years) who had migrated from a malaria-free area in Indonesia (classified as a nonimmune population) received 3 months of weekly prophylaxis with chloroquine. Serum samples were collected from August 1996 through July 1999. Samples were obtained at the time of arrival in Papua, every 2 months thereafter, and at the time of diagnosis for each symptomatic malaria infection. Informed consent was obtained from the participants or their parents or guardians. Malaria infections were diagnosed from blood smears obtained every 2 weeks or whenever volunteers were symptomatic. Participants were visited 3 times per week by health workers and actively questioned regarding the presence of symptoms. Serum was obtained from blood samples and stored at −80°C until use.

A subset of serum samples collected from 24 individuals was chosen for the invasion-inhibition assay (table 1). The selection criteria were as follows: (1) *P. falciparum* infection (5 sets of samples from individuals who had a single infection and 19 sets from individuals who had multiple infections); (2) ⩾1 serum samples obtained during serial infection that had a high optical density (OD) value (i.e., greater than the median value of 0.77) for $MSP1_{19}$ (Wellcome allele) as measured by ELISA. Every set of serum samples consisted of a sample obtained prior to infection and routine samples collected during a convalescence period following each sequential infection. A second set of serum samples from 10 individuals who had no history of *P. falciparum* infection were used as negative controls. Pooled serum samples collected from 22 individuals with documented *P. falciparum* infection living in Madang, Papua New Guinea, were used as positive controls [15].

ELISA. The total anti-$MSP1_{19}$ Ig level was measured by ELISA, performed as described elsewhere [15]. In brief, $MSP1_{19}$–glutathione S-transferase (GST) recombinant protein fusions (Wellcome and MAD20) were applied to 96-well microtiter plates and serum samples were measured at a 1:1000 dilution. Specific optical density values were calculated by subtracting the control optical density values measured on GST alone, and positive serum samples were defined as those that gave a specific optical density value greater than the mean plus 3 standard deviations of optical density values obtained for 30 control serum samples obtained from individuals who had never been exposed to malaria. Isotype-specific ELISA was also performed to measure IgG1, IgG2, IgG3, IgG4, and IgM levels for samples that gave positive results for total Ig [15].

Invasion-inhibition assay. Invasion-inhibition assays were performed as described elsewhere by using *P. falciparum* parasite lines D10-*Pf*M3' ($MSP1_{19}$ MAD20 allele) and D10-*Pc*MEGF (*P. chabaudi* $MSP1_{19}$) [10]. In brief, ring-stage parasites were syn-

Table 1. Specific inhibitory activity and total Ig responses against the 19 kDa C-terminal fragment of *Plasmodium falciparum* merozoite surface protein 1 observed in 24 subjects from northwestern Papua, Indonesia.

Subject	Age, years	Time serum sample obtained: Prior to infection	After infection, by infection no.: 1	2	3	4	5
		Inhibition activity					
CQ005	25	NSI	NSI[a]				
CQ061	30	NSI[b]	NSI[b]				
MF050	32	NSI	NSI[b]				
CQ054	33	NSI[b]	NSI[b]	NSI[b]			
MF099	36	NSI	SI[b]	SI[b]			
CQ010	37	NSI[b]	NSI[b]	SI[b]	SI[b]		
MF010	34	NSI	NSI[b]	NSI[b]	NSI[b]		
MF038	40	NSI[b]	E[b]	SI[b]	SI[b]		
MF048	30	NSI[b]	SI[b]	SI[b]			
MF077	30	NSI	SI[b]	NSI[b]	NSI[b]		
CQ062	25	NSI[b]	NSI[b]	NSI[b]	NSI[b]	NSI[b]	
MF117	36	NSI	NSI	NSI[b]	NSI[b]	NSI[b]	
MF060	30	NSI[b]	NSI[b]	NSI[b]	SI[b]	SI[b]	
MF112	40	NSI[b]	NSI[b]	NSI[b]	SI[b]	SI[b]	SI[b]
CQ095	12	NSI	NSI[b]				
MF074	7	NSI	NSI[b]				
MF108	12	NSI	E[b]	SI[a]			
MF125	8	NSI	NSI[b]	SI[b]			
MF051	7	SI[b]	NSI[b]	SI[b]			
MF053	9	NSI	NSI[b]	NSI[b]	NSI[a]		
MF090	10	NSI[b]	NSI[b]	SI[b]	NSI[b]		
MF030	10	NSI[b]	NSI[b]	NSI[b]	NSI[b]		
MF105	6	NSI	NSI[b]	E[b]	NSI[b]		
CQ105	10	NSI	NSI[b]	NSI[b]	NSI[b]		
		Proportion positive, no. of subjects					
Ig		11/24	22/23	18/18	12/12	4/4	1/1
Inhibition		1/24	3/22	8/18	4/13	2/4	1/1

NOTE. E, excluded due to failure of schizont rupture; NSI, no significant inhibition (inhibition activity <15% or $P > .05$); SI, significant inhibition (inhibition activity >15% and $P < .05$).

[a] ELISA Ig result not available.

[b] Ig-positive by ELISA.

chronized by use of sorbitol lysis and inhibition assays were conducted at 24 h, when parasites reached schizont stage. Into each well, 50μL of infected red blood cell (RBCs) (at 1% parasitemia and 4% hematocrit) and 50μL of heat-inactivated serum (1:5 dilution) were added (final serum dilution, 1:10). Serum samples were tested on both lines in triplicate. Human nonimmune serum ([HNIS] negative control), rabbit anti–*Pc*MSP1$_{19}$ antiserum, and rabbit anti–*Pf*MSP1$_{19}$ antiserum (positive controls) were included in every assay. When all schizonts had ruptured (~26 h), blood smears were made, and the number of ring-infected RBCs/1000 RBCs was counted. From triplicate wells, the mean parasitemia level was calculated, and invasion was expressed as a percentage of the mean parasitemia level in parallel cultures of each parasite line in the presence of pooled HNIS. The net invasion inhibition attributable to *Pf*MSP1$_{19}$-specific antibodies was calculated by subtracting the inhibition identified in the D10-*Pc*MEGF cultures from the inhibition identified in the D10-*Pf*M3' cultures.

Statistical analysis. The Student's *t* test was used to compare the 2 parasite lines with respect to the parasite invasion rate in the presence of a particular serum. Inhibitory activity was considered positive when $P < .05$ and net inhibition >15%. Mann-Whitney *U* tests were used to compare invasion rates and inhibition levels between groups. Spearman rank correlation tests were used to assess the correlation between the level of antibody to MSP1$_{19}$ and the invasion inhibition percentage. The χ^2 test was used to examine the association between antibody level and inhibitory activity, and the survival analysis test was used to determine whether high inhibitory activity delayed the occurrence of subsequent reinfection with *P. falciparum*.

RESULTS

Total anti-MSP1$_{19}$ Ig responses measured by ELISA. The ELISA data initially used to select the serum samples to be tested in the invasion-inhibition assay were obtained by using the MSP1$_{19}$ Wellcome allele (Q-KNG), whereas the *P. falciparum* line D10-*Pf*M3' used in the assay expresses the MAD20 allele (E-TSR). To determine whether the allelic type affected the antibody responses to MSP1$_{19}$, the ELISA was repeated for the 92 serum samples against the 2 MSP1$_{19}$ alleles in parallel. The ELISA results for the MAD20 allele showed that 71 of 92 samples (77%) were obtained from positive responders, compared with 58 of 92 samples (64%) obtained from positive responders to the Wellcome allele. A significant correlation was observed between the total Ig responses to the 2 MSP1$_{19}$ alleles ($r = 0.96$; $P < .001$). For further analysis, the MAD20 ELISA data were used.

Most of the participants in this study (22 of 23) showed a positive anti-MSP1$_{19}$ antibody response after their first *P. falciparum* infection, and the response remained positive following subsequent infections (table 1). However, it appeared that 11 (46%) of 24 participants had preexisting antibody prior to their first infection that occurred in Armopa and that other individuals in this study (3 [30%] of 10) who were not infected with *P. falciparum* also had low levels of anti-MSP1$_{19}$ antibodies. This indicates that some individuals may have experienced *P. falciparum* infection(s) prior to their migration to Armopa, despite a negative history obtained on enrolment.

MSP1$_{19}$-specific invasion-inhibitory activity measured by invasion inhibition assay. MSP1$_{19}$-specific invasion-inhibitory activity was evaluated by using the previously described *P. falciparum* lines D10-*Pf*M3' and D10-*Pc*MEGF, which are isogenic except for their MSP1$_{19}$ domains [10]. In the presence of serum obtained prior

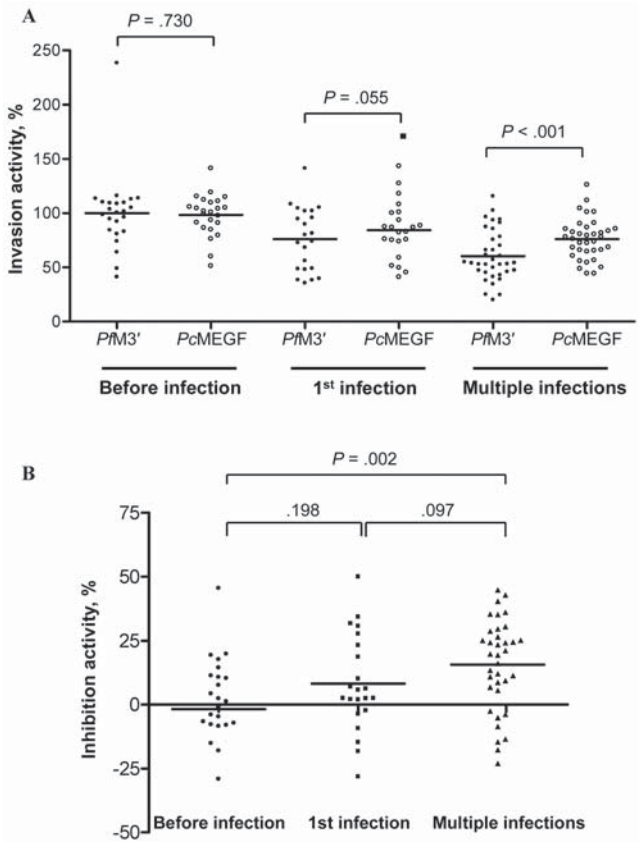

Fig 1. *A,* Invasion activity against 2 isogenic *Plasmodium falciparum* lines (*Pf*M3' and *Pc*MEGF) for 24 serum samples obtained prior to infection, 22 serum samples obtained after first infection, and 36 serum samples obtained after multiple infections. Invasion activity is expressed as a percentage of the invasion activity observed in nonimmune serum samples. *Dots,* values of individual serum samples; *horizontal lines,* means. *P* values were calculated by using paired Student's *t* tests. *B,* Comparison of the *Pf*MSP1$_{19}$-specific invasion inhibition between these 3 groups of serum samples, calculated by subtracting the inhibition activity identified in the D10-*Pc*MEGF cultures from the inhibition obtained with D10-*Pf*M3'. *Dots,* values of individual serum samples; *horizontal lines,* means. For the preinfection group, 1 serum sample with inhibition activity of −97% is not shown. *P* values were calculated by using the Mann-Whitney *U* test.

to infection, invasion activity for both D10-*Pf*M3' and D10-*Pc*MEGF was similar (figure 1*A*). The mean invasion activity level (expressed as a percentage of the invasion observed in nonimmune serum samples) was 98.2% for *Pc*MEGF and 100.6% for D10-*Pf*M3' ($P = .730$). After the first infection, the overall mean invasion activity observed for all serum samples remained nonsignificant when comparing the 2 parasite lines, being 84.2% for *Pc*MEGF and 76.0% for *Pf*M3' ($P = .055$) (figure 1*A*). However, when examined individually, 3 serum samples (obtained after the first infection from subjects MF048, MF077, and MF099) (table 1) conferred significantly greater invasion inhibitory activities for *Pf*M3' than for *Pc*MEGF, with differences in inhibition percentage between the 2 strains of 30%–50%. The invasion-inhibitory activity against the *Pf*M3' strain increased further after the second malaria infection. The mean parasite invasion rates observed for *Pc*MEGF and *Pf*M3' in the presence of serum were 76% and 60%, respectively, resulting in a specific inhibition percentage against *Pf*MSP1$_{19}$ of 16% ($P < .001$) (figure 1*A*. In general, the invasion-inhibitory activity in serum samples obtained ⩾2 infections was significantly higher than that in serum samples obtained prior to infection ($P = .002$) (figure 1*B*).

Across the population, MSP1$_{19}$-specific inhibition activity ranged from −97% to 46%, with positive inhibition (i.e., inhibition >15% and $P < .05$) being 15%–46%. For most individuals who generated specific inhibitory antibodies against MSP1$_{19}$, it appeared that the specific inhibitory response to MSP1$_{19}$ began after the second *P. falciparum* infection (table 1). Repeated infections resulted in either stable or enhanced inhibition against MSP1$_{19}$, with specific activities ranging between 19% and 43%. It should be noted that 3 of the 92 serum samples used in the assay (1 sample each from subjects MF108, MF038, and MF105) were excluded from the statistical analyses, as their inhibitory activity was found to be caused by retarded intraerythrocytic parasite development and failure of schizont rupture rather than inhibition of merozoite invasion. When sets of serum samples with at least 2 sequential infections were analyzed individually, various kinetics of the acquisition of invasion-inhibitory activity were observed. Eight subjects failed to produce inhibitory activity during their infections (CQ054, MF010, CQ062, MF117, MF053, MF030, MF105, and CQ105), whereas 7 individuals generated stable inhibitory activity after the second infection (CQ010, MF038, MF048, MF099, MF108, MF125, and MF051), and 2 individuals generated stable inhibitory activity after the third infection (MF060 and MF112) (table 1 and figure 2). For 1 individual who developed inhibitory antibodies after the first infection (MF077) and 1 individual who developed them after the second infection (MF090), the inhibitory antibodies disappeared after further infections (table 1)).

Relationship between MSP1$_{19}$-specific Ig and invasion inhibitory activity. The total number of samples found to be Ig-positive by ELISA and found to have significant invasion-inhibitory activity was 18 of 68 (28%). The Spearman rank test revealed a significant correlation ($r = 0.4842$; $P < .001$) between inhibitory antibodies and the total Ig response to MSP1$_{19}$. There was also a significant correlation between inhibitory antibodies and the total IgG response ($r = 0.3747$; $P = .002$), with IgG1 as the predominant antibody subclass.

To further examine the correlation between anti-MSP1$_{19}$ antibody level and the invasion inhibitory activity, all serum samples were ranked in order of ELISA optical density values and divided into quartiles. The χ^2 test showed that the group with the highest optical density values (>75% quartile) had significantly higher inhibitory activity than the groups with lower optical density values (<25% quartile and 25%–50% quartile), and the

Table 2. Correlation between Ig response to the 19 kDa C-terminal fragment of *Plasmodium falciparum* merozoite surface protein 1 and invasion-inhibitory activity

	Q1	Q2	Q3	Q4
Inhibitory samples/total samples, no.[a,b]	0/23	2/22	8/22	10/22
P[c]				
Comparison to Q1		.157	**.007**	**.003**
Comparison to Q2			.157	**.039**
Comparison to Q3				.691

NOTE. Samples were ranked in order of optical density value and divided into quartiles: first quartile (Q1), <25%; second quartile (Q2), 25%–50%; third quartile (Q3), >50%–75%; fourth quartile (Q4), >75%.

[a] One sample was excluded in Q2, Q3, and Q4 owing to failure of schizont rupture.

[b] Samples with significant inhibition (inhibition activity >15% and $P < .05$).

[c] By the χ^2 test; significant values are in bold type.

>50%–75% quartile group also had significantly higher inhibitory activity than the <25% quartile group (table 2).

Relationship between MSP1$_{19}$-specific invasion-inhibitory activity and immunity to reinfection. Serial infections in individual subjects were separated by a measured number of days. We set out to determine whether the degree of invasion-inhibitory activity correlated with the interval between infections. We analyzed subjects' infection-free intervals to determine whether higher levels of inhibitory antibodies delayed the time to reinfection with *P. falciparum* (figure 3). For this analysis, samples from subjects who had had serial *P. falciparum* infections (82 samples) were ranked in the order of inhibitory antibody level from least to greatest, and only the top and bottom quartiles were used in the analysis. Although at the end of the study, the group with higher inhibitory antibody levels had a higher proportion of subjects who remained uninfected, the median rate of the 2 groups being compared (the group with values <25% quartile and the group with values >75% quartile) were not significantly different ($P = .204$).

DISCUSSION

Epidemiological surveys reveal that immunity to malaria is acquired slowly, reaching its greatest level only after a number of years of residence in areas where malaria is endemic and after a number of infections [16, 17]. Numerous studies have examined responses to particular proteins and the correlation of these responses with the immune state [18–21]. Many of these studies conclude that the particular protein under study is associated with protection because antibody responses to it increase over time; however, it has been difficult to demonstrate functionally protective activity associated with antibody responses to any given protein, partly because of the difficulties of performing the assays and assigning functional effects to specific proteins.

The development of paired transgenic lines that differ only in the C-terminus of MSP1 has provided a tool capable of measuring one aspect of antibody function: invasion inhibition [10]. Keenihan et al. [22] have examined this population by using conventional ELISA and showed that the anti-MSP1$_{19}$ IgG response appeared after the first infection and increased gradually as cumulative exposure increased. We investigated the kinetics of acquisition of functional antibody to the same target and showed that of the 24 individuals infected with *P. falciparum,* only 9 had acquired MSP1$_{19}$-specific invasion-inhibitory activity by the end of the study period. Of those who acquired inhibitory activity, most required at least one additional infection, if not more, to elicit specific inhibitory antibodies against MSP1$_{19}$. This provides an interesting and unexplained contrast with the observation involving a group of Australian travellers who acquired a malaria infection after many years of residence in an area with no malaria transmission [23]. In that study group, 45% of subjects showed significant growth-inhibitory activity after their first infection. The reason for this significant difference ($P < .05$) from what was observed in the transmigrants involved in the present study is not known. The mean age of the transmigrants was 22.8 years, whereas it was 34 years for the Australian travelers. Although the capacity to develop an effective antibody response after infection is age-dependent [24], this is unlikely to be the entire reason for the observations reported here.

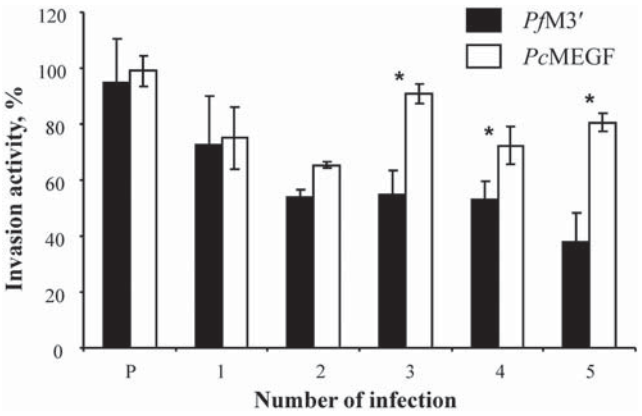

Fig 2. Example of parasite invasion activity for 2 isogenic *Plasmodium falciparum* transfectant lines (*Pf*M3' and *Pc*MEGF) in the presence of serum samples from an immune individual. Samples were taken from patient MF112, who experienced 5 sequential *Plasmodium* infections during the 18-month study period. Significant MSP1$_{19}$-specific inhibition activity was acquired after the third infection and was maintained and enhanced after subsequent infections. Asterisks indicate significant inhibition ($P < .05$, by paired Student's *t* tests and net inhibition activity >15%). Error bars indicate standard deviations. Invasion activity is expressed as a percentage of the invasion activity observed in nonimmune serum samples.

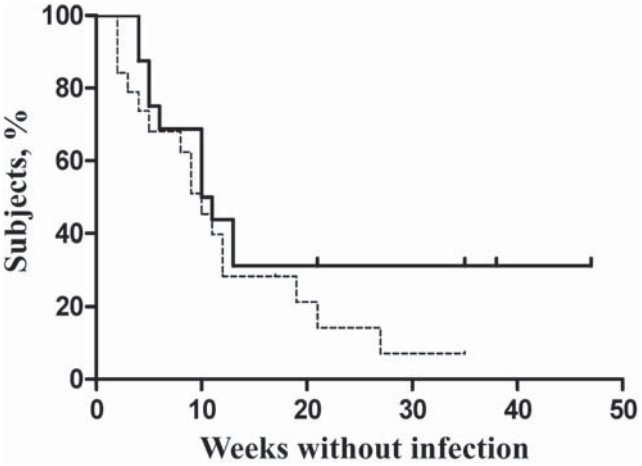

Fig 3. Duration of infection-free intervals for individuals who had serial *Plasmodium falciparum* infection. *Dashed line,* individuals with inhibitory antibody levels in the lowest quartile; *solid line,* individuals with inhibitory antibody levels in the highest quartile. The median infection-free survival time of individuals with antibody levels in the lowest and highest quartiles was 10.0 and 10.4 weeks, respectively ($P = .204$).

Another difference between this study population and that of the Eisen et al. study [23] is ethnicity, which may reflect differences in immune response genes, and consequently, in the quality of the acquired immune response. We do not have major histocompatibility complex or other genotyping data for either population, and at present there is relatively little evidence to support this explanation. Alternatively, residence in an endemic area with a higher burden of other diseases, including helminth infection, may act as an immune suppressor or modulator. There is some evidence for the view that such infections may favor Th2 responses that would decrease cytophilic antibody responses and decrease effective immunity [25, 26]. However, because invasion inhibition is likely to be related to fragment variable–binding specificity and unlikely to be affected by antibody isotype because of the absence of serum or cells in the inhibition assay, there is as yet no described mechanism by which a Th2 switch would decrease inhibition. It should be noted that previous studies involving travelers have reported a relatively higher rate for the acquisition of functional antibodies, specifically transmission-blocking antibodies to *Pfs*45/48, than the rate found in malaria-endemic populations [27], so this difference in rate of acquisition of functional antibody may be more general, suggesting a global difference in immune responsiveness.

When we looked at our study population over time, we found that specific inhibitory activity could decline or disappear after reaching significant levels. Two individuals showed inhibitory activity at the first time point but subsequently lost it (table 1), which reinforces observations of the short-lived nature of immunity in malaria-endemic populations [16, 17]. In addition, several people acquired new infections after the development of inhibitory antibodies to $MSP1_{19}$. One possible explanation might be the development of strain-specific immunity that allowed infection with parasites expressing variant $MSP1_{19}$ alleles during later infections [28]. It is equally possible that certain alleles of $MSP1_{19}$ circulating in nature may induce antibodies that react poorly with the specific allele present in the test strains, thus failing to show inhibitory activity in the invasion-inhibition assay even though the patient had inhibitory antibodies to other alleles. We note that antibodies in these study serum samples cross-reacted with 2 of the 4 known $MSP1_{19}$ sequence variants, E-TSR (MAD20) and Q-KNG (Wellcome), but this cross-reactivity may not extend to inhibitory activity.

In contrast to the epidemiological study in Kenya that showed that $MSP1_{19}$-specific inhibitory activity, particularly in those with activity levels >75% quartile, was associated with resistance to *P. falciparum* infection [11], our study found that having *Pf*$MSP1_{19}$-specific inhibitory activity levels >75% quartile did not prevent individuals from being reinfected with *P. falciparum,* and in some individuals, inhibitory activity was not acquired even after several infections. This finding is not surprising, because the subjects recruited in this study were nonimmune individuals who had been present in this malaria-endemic area for less than 18 months; the population in the John et al. study [11] were Kenyan residents with a long history exposure to repeated infection. Furthermore, the inhibitory activity levels generated by the participants in this study were lower, compared with those observed in the Kenya study. A previous study [10] using serum samples obtained from lifelong residents in Papua New Guinea who lived close to the transmigrant settlement showed higher inhibitory responses (average inhibition activity level, ~25%), compared with those observed in the present study (average inhibition activity level of ~16% after multiple infections). A lack of association between $MSP1_{19}$-specific antibodies and resistance to parasitemia has been reported in a cross-sectional survey in The Gambia [29]. Although the inhibitory activity level measured in vitro serves as a more accurate correlate of protection than total Ig measurements, it may not be a complete predictor of immune status in vivo because noninhibitory antibodies can also contribute to protection through fragment crystallizable region–mediated pathways, as demonstrated by McIntosh et al. [30].

When the data presented above is taken together, it seems that extended exposure to *P. falciparum* infection is needed to generate a higher functional inhibitory response against $MSP1_{19}$ that is capable of preventing subsequent infection. Multiple and extended natural malaria exposures will also generate immune responses that are not solely directed toward a specific antigen; instead, they will generate inhibitory antibodies to other targets that could work together. In the present study, we used the absence of detectable parasitemia as an operational definition indicating that the subject had some ability to resist infection. The immune mechanisms operating to provide this level of resistance may be different from those that operate to establish a state of clinical immunity. A clinically immune patient may become infected but does not respond with signs such as fever. Studies of this transmigrant group showed that clinical immunity

required at least 4 infections within 12–24 months of entering the malaria-endemic area, and by then the individual would likely have developed some level of growth-inhibitory antibodies to $MSP1_{19}$ [22].

Overall, the findings obtained in this investigation have enriched our understanding of the kinetics of acquisition of antimalarial immune responses. It will be interesting to determine whether immunization with recombinant $MSP1_{19}$ in the form of a subunit vaccine will be capable of inducing inhibitory antibodies in malaria-endemic populations.

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