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Evidence That Invasion-Inhibitory Antibodies Specific for the 19-kDa Fragment of Merozoite Surface Protein-1 ($MSP\text{-}1_{19}$) Can Play a Protective Role against Blood-Stage *Plasmodium falciparum* Infection in Individuals in a Malaria Endemic Area of Africa[1]

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The C-terminal 19-kDa fragment of *Plasmodium falciparum* merozoite surface protein-1 ($MSP\text{-}1_{19}$) is a target of protective Abs against blood-stage infection and a leading candidate for inclusion in a human malaria vaccine. However, the precise role, relative importance, and mechanism of action of Abs that target this protein remain unclear. To examine the potential protective role of Abs to $MSP\text{-}1_{19}$ in individuals naturally exposed to malaria, we conducted a treatment time to infection study over a 10-wk period in 76 residents of a highland area of western Kenya during a malaria epidemic. These semi-immune individuals were not all equally susceptible to reinfection with *P. falciparum* following drug cure. Using a new neutralization assay based on transgenic *P. falciparum* expressing the *P. chabaudi* $MSP\text{-}1_{19}$ orthologue, individuals with high-level $MSP\text{-}1_{19}$-specific invasion-inhibitory Abs (>75th percentile) had a 66% reduction in the risk of blood-stage infection relative to others in the population (95% confidence interval, 3–88%). In contrast, high levels of $MSP\text{-}1_{19}$ IgG or IgG subclass Abs measured by enzyme immunoassay with six different recombinant $MSP\text{-}1_{19}$ Ags did not correlate with protection from infection. IgG Abs measured by serology and functional invasion-inhibitory activity did not correlate with each other. These findings implicate an important protective role for $MSP\text{-}1_{19}$-specific invasion inhibitory Abs in immunity to blood-stage *P. falciparum* infection, and suggest that the measurement of $MSP\text{-}1_{19}$ specific inhibitory Abs may serve as an accurate correlate of protection in clinical trials of MSP-1-based vaccines. ***The Journal of Immunology*, 2004, 173: 666–672.**

Falciparum malaria exacts an immense burden on the health of residents of sub-Saharan Africa (1). Antimalarial drugs and insecticide impregnated bed nets have had an impact on malaria morbidity but these approaches have financial, logistic, and biologic limitations. A vaccine against *Plasmodium falciparum* is thus highly desirable from a public health perspective (2). The rationale for selecting vaccine Ags specific for blood-stage *P. falciparum* is based on the premise that they will enhance host immune responses that interfere with the repeated cycles of invasion and parasite growth in RBC, thereby diminishing the level of asexual parasitemia and morbidity associated with cytoadherence of infected RBC to endothelial cells (3).

The multiple malaria infections experienced by residents of areas of Africa where transmission is perennial or has predictable seasonal fluctuations generally result in decreased prevalence and density of asexual parasitemia and less morbidity in adults relative to infants (4, 5). These age-related changes are less pronounced in highland areas where transmission is episodic (6, 7). T cell and B cell immunity are important in vaccine-induced resistance against blood-stage *P. falciparum* (8, 9), and several lines of evidence indicate that Abs to merozoite surface proteins (MSP)[4] elicited by natural infection mediate the nonsterilizing immunity that characteristically develops in many residents of malaria endemic areas. Adoptive transfer of the Ig fraction of pooled sera obtained from malaria-immune adult Africans to malaria-infected children leads to dramatic, albeit transient, decreases in the level of asexual parasitemia (10–13). Sera from persons with recent malaria infection impair the growth of *P. falciparum* in vitro, although the magnitude of this inhibition is variable (14–16). MSP-1 is one of several targets of growth-inhibitory Abs elicited by natural infection (15, 17), and based on studies of vaccine studies of mouse models (18, 19) and *Aotus* monkeys (20–22) challenged with *P. falciparum*, is a leading candidate for inclusion in a human blood-stage vaccine. MSP-1 is expressed initially as a ~200-kDa protein during the late stages of erythrocytic schizogony. Following rupture of the schizont and release of merozoites into the plasma, a 42-kDa and ultimately 19-kDa fragment of MSP-1 ($MSP\text{-}1_{19}$) is produced and carried into the newly infected erythrocyte during the process of

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[4] Abbreviations used in this paper: MSP, merozoite surface protein; $MSP\text{-}1_{19}$, 19-kDa fragment of MSP-1; EGF, epidermal growth factor; IIA, invasion-inhibitory Ab.

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invasion (23). Considerable evidence indicates that the two cysteine-rich epidermal growth factor (EGF)-like domains included in MSP-1_{19} have an essential role in blood-stage growth. These domains appear to be prime targets of protective immune responses (24, 25).

Participants in vaccine trials in malaria endemic areas cannot be deliberately challenged with *P. falciparum* on practical and ethical grounds. A surrogate of blood-stage immunity under conditions of natural transmission would thus facilitate the screening and evaluation of MSP-1 in anticipated vaccine trials. In addition, because clinical morbidity specifically attributable to malaria is infrequent even in areas where transmission is high, a quantitative functional assay of MSP-1 related immunity might reduce the number of participants needed to achieve statistical power to detect significant differences between vaccinated and control groups. Some (but not all) population-based surveys have shown that IgG Abs to MSP-1_{19}, particularly those of the IgG1 and IgG3 subclasses, correlate inversely with the incidence and density of asexual parasitemia and frequency of uncomplicated morbidity (26–30). This correlation is however imprecise, possibly because recombinant MSP-1_{19} proteins used in serologic assays may not have conformations similar to the tertiary structure of the native molecule and because polyclonal responses elicited by natural infection may not include Abs with specificities that directly interfere with MSP-1-related function or that impair processing of the 42-kDa to the 19-kDa fragment that is essential for invasion (31–33). Egan et al. (15) first demonstrated that invasion-inhibitory Abs (IIA) specific for MSP-1_{19} were present in IgG affinity purified from pooled sera of west African adults. The recent development of a transgenic *P. falciparum* line in which the region of the native *msp1* gene sequence encoding MSP-1_{19} is replaced with the orthologue of the distantly related mouse malaria *P. chabaudi* has enabled quantification of Abs that have *P. falciparum* MSP-1_{19} (PfMSP-1_{19})-specific IIA (34). Taking advantage of this newly developed in vitro assay, we aimed to determine in a field setting in the highlands of western Kenya whether the functionally relevant IIA that recognize this molecule are a better predictor of protection from blood-stage parasitemia than are MSP-1_{19}-specific IgG Abs measured by standard enzyme immunoassays (ELISA). The study was conducted in a highland, epidemic-prone area during a malaria outbreak as part of a larger study investigating correlates of protective immunity in individuals residing in an area of unstable, episodic malaria transmission. Adults and children were enrolled in the study, as studies in other epidemic-prone highland areas have demonstrated that adults and children in these areas are equally susceptible to infection and disease with *P. falciparum* (6).

Materials and Methods

Study design and subjects

Review and approval of the study was obtained from the Kenya Medical Research Institute National Ethical Review Committee and the Institutional Review Board for Human Studies at University Hospitals of Cleveland, Case Western Reserve University (Cleveland, OH). Written informed consent was obtained from all participants aged 16 years or older and from guardians of participants younger than this age.

Participants were recruited from residents of Kabobo, an isolated village located at an altitude of 2134 meters in the Uasin Gishu district in Kenya. Transmission of *P. falciparum* in this highland area is episodic and does not occur on a regular seasonal basis. Epidemics of malaria in various highland areas of western Kenya were first reported in the early to mid-20th century (7, 35, 36). Malaria infection surveys conducted in Kabobo during periods of no or little rainfall have shown that the prevalence of blood-stage *P. falciparum* in symptom-free 5- to 10-year-old school children is 8.9%. The prevalence increased to 45.5% during a period of heavy rainfall (37, 38).

The treatment-infection study commenced in May 1997, at the end of a 6-mo period of little rainfall, when increasing rain related to an El Niño event led to an epidemic in the area. Inclusion criteria included life-long residency in Kabobo, inhabiting a local domicile for the previous year, and lack of malaria morbidity, e.g., fever, chills, myalgia, or other self-identified illnesses within the previous 2 wk. Exclusion criteria were pregnancy and use of antimalarial drugs within the previous 2 wk. Recruitment was done after a series of community and individual meetings were held to explain the nature and purpose of the study. Persons agreeing to participate were given a single dose of sulfadoxine-pyrimethamine (300 mg), the government recommended drug for treatment of uncomplicated malaria in Kenya in 1997. Blood for laboratory studies (see below) was obtained by venipuncture before treatment with sulfadoxine-pyrimethamine. Subjects were followed weekly for the next 12 wk for symptoms of malaria morbidity and diagnosis of malaria infection by microscopic inspection of blood smears obtained by pricking the finger with a lancet (see below). Health care workers remained in the area during the entire study period. Clearance of parasitemia was documented 2 wk after sulfadoxine-pyrimethamine administration, and time to reinfection was calculated as time to infection after parasite clearance, leaving a total of 10 wk of follow-up time.

We intended to obtain blood immediately from persons who self-identified with fever, chills, or any symptoms consistent with malaria. A single dose of sulfadoxine-pyrimethamine was to be given when a positive blood smear was observed. Persons with positive blood smears within 2 wk of administration of the initial dose of sulfadoxine-pyrimethamine were given quinine plus doxycycline and excluded from further analysis. A total of 84 subjects (34 children ages 1–8 years old, 50 adults ages 16–80 years old) were recruited.

Laboratory studies

Malaria infection was diagnosed by microscopic inspection of thick and thin blood smears. Blood smears were prepared and stained with Giemsa, and slides examined by two experienced microscopists used by the Division of Vector Borne Diseases of the Kenya Ministry of Health. The microscopists were blinded to the study protocol and read each of the slides twice. A smear was deemed negative when no parasites were observed after counting microscopic fields that included at least 200 leukocytes. The density of parasitemia was expressed as the number of asexual *P. falciparum* per microliter of blood assuming a leukocyte count of 8000/μl. Malaria species identification was made by inspection of thin blood smears.

Abs to MSP-1_{19} were measured by ELISA with plasma diluted 1/100 and 10 ng/ml protein applied to microtitration plates (15, 37). Recombinant MSP-1_{19} proteins corresponding to the four major described variants of MSP-1_{19} (the Wellcome/K1 (E-TSR) and MAD20 (Q-KNG) alleles as well as the E-KNG and Q-TSR variants) were expressed in *Saccharomyces cerivisiae* (39), obtained from the Malaria Research and Reference Reagent Resource Center (Manassas, VA). The Wellcome/K1 and MAD20 alleles were also expressed in *Escherichia coli* as GST fusion proteins (40). Ab levels were expressed in arbitrary units, which were calculated by dividing the OD generated by the test sample by the mean OD + 3 SD generated by samples from 40 North American or Australian individuals who had never been exposed to malaria. Values that were ≥1.0 arbitrary units were considered positive. OD values representing 1 arbitrary unit for IgG, IgG1, IgG2, IgG3, and IgG4 Abs to the MAD20 variant of MSP-1_{19} expressed in *S. cerivisiae* were 0.138, 0.157, 0.213, 0.070, and 0.105, respectively.

MSP-1_{19}-specific IIA was performed using D10 *P. falciparum* (which encodes the MAD20 allele) and an isogenic D10-*P. chabaudi* mouse EGF (PcMEGF) parasite line in which the *P. chabaudi* orthologue replaces the region of *P. falciparum msp1* encoding the two EGF-like domains of MSP-1_{19} (34). Ring-stage parasites were synchronized twice by sorbitol lysis and allowed to mature to trophozoite/schizont stages. Purified parasites were adjusted to 4% hematocrit with 1% infected RBC, and 50 μl of aliquots were placed in 96-well microtitration plates with an equal volume of 1/10 prediluted plasma in culture medium (final sera dilution 1/20). The same batch of prediluted plasma was added to the two parasite lines in the same assay. The cultures were incubated for 26 h to allow for schizont rupture and merozoite invasion. Thin smears were made, fixed with methanol, and stained with Giemsa. The number of ring-stage parasites per 500 (assay 1) or 1000 (assay 2) RBC were counted. The mean parasitemia for duplicate wells was calculated and results expressed as a percentage of the parasitemia in parallel cultures that contained nonimmune sera from people who had not been exposed to malaria. The percentage of invasion-inhibition specifically due to anti-MSP-1_{19} Ab (MSP-1_{19} IIA) was calculated with the following formula: percentage of invasion of D10 PcMEGF parasites relative to nonimmune controls − percentage of invasion of D10 isogenic parasites relative to nonimmune controls.

Statistics

Differences in the proportion of individuals with Abs to MSP-1_{19} determined by serology (ELISA) were evaluated by the χ^2 test. The significance of differences in Ab levels was evaluated by the nonparametric Mann-Whitney *U* test. Correlations between continuous variables, e.g., Ab levels to the two alleles of MSP-1_{19}, were assessed by Spearman's rank correlation.

Kaplan-Meier survival analysis was used to compare the time to development of *P. falciparum* parasitemia in weeks according to whether an individual had high-level or low-level MSP-1_{19}-specific IgG Abs (as measured by ELISA) or high-level or low-level MSP-1_{19}-specific IIA activity. For assessment of time to infection, levels of IgG Abs to the MAD20 variant of MSP-1_{19} expressed in *S. cerivisiae* were used. Risk of infection after administration of sulfadoxine-pyrimethamine at the beginning of the study was assessed by Cox proportional hazards analysis. Age and malaria infection status before sulfadoxine-pyrimethamine were adjusted for in the final Cox model. We hypothesized that a high level of MSP-1_{19} IgG Abs or a high degree of PfMSP-1_{19}-specific IIA activity would correlate most strongly with protection from infection and therefore chose the 75th percentile of overall IgG Abs or IIA of the group to distinguish high vs low activity. An initial sample size of 100 individuals was calculated to give the study 85% power to detect a 60% decrease in the risk of infection in individuals with high-level Abs or IIA (>75th percentile), assuming an infection rate of 60% in the baseline (low activity) group. Our final sample size in the treatment-reinfection study, after exclusion of individuals who did not wish to have blood samples taken for malaria smear testing, individuals with persistent infection, and individuals with insufficient serum for testing, was 76 persons. We calculated that with this sample size the study had 71% power to detect a 60% decrease in risk of infection assuming an infection rate of 60% in the baseline group.

Results

Study design, infection status, and outcome

Eight of 84 people recruited to participate were excluded from analysis. They developed positive blood smears within 2 wk of taking sulfadoxine-pyrimethamine (indicative of liver-stage infection at the time of drug administration) because blood-stage *P. falciparum* was not completely eliminated (two individuals) or because insufficient plasma was obtained (six individuals). Thirteen of the remaining 76 persons (17.1%) included in the analysis of time to infection had blood-stage *P. falciparum* infection before administration of sulfadoxine-pyrimethamine.

Thirty-four of 76 (44.7%) participants developed blood-stage *P. falciparum* infection 1–10 wk after documented clearance of blood-stage infection with sulfadoxine-pyrimethamine. None of the participants, irrespective of whether they had blood-stage infection, had fever or other symptoms of malaria morbidity. The range of densities was from 40 to 600 asexual *P. falciparum* per microliter of blood. Infection with other *Plasmodium* species was not observed. Age, gender, and pretreatment infection status had no significant effect on the time to or risk of infection (Cox proportional hazards analysis; data not shown).

Abs to recombinant MSP-1_{19} proteins measured by ELISA

MSP-1_{19}-specific IgG Ab reacting with a yeast-expressed recombinant MSP-1_{19} representing the MAD20 allele was detected in plasma from 32 of 76 (42.1%) individuals immediately before drug administration. There was a bias toward IgG1 and IgG3 subclass Abs although all four subclasses were detected. IgG1, IgG2, IgG3, and IgG4 Abs were detected in 25 (32.9%), 11 (14.5%), 24 (31.6%), and 12 (15.8%) persons, respectively. There was no difference in the prevalence or level of IgG or IgG1 and IgG3 subclass Abs among participants 8 years of age and younger vs those 16 years or older. The prevalence of IgG2 and IgG4 Abs was higher in the younger age group, e.g., 26.7 and 30.0% of subjects 8-years-old or younger vs 6.5% for both IgG2 and IgG4 for subjects 16 years of age or older ($p < 0.05$ using χ^2 and Wilcoxon rank-sum analysis).

The 76 samples were tested against yeast-expressed recombinant MSP-1_{19} representing the Wellcome/K1 and MAD20 alleles. IgG Abs to the two variants correlated strongly with each other ($\rho = 0.94$, $p < 0.0001$; Fig. 1*A*). There was also strong concordance for IgG Ab reactivity with the MAD20 and Wellcome/K1 alleles when recombinant MSP-1_{19} was expressed in *E. coli* as GST fusion proteins ($\rho = 0.94$, $p < 0.0001$; data not shown). Ab reactivity with the MSP-1_{19} expressed in yeast and *E. coli* correlated with each other, but not as well as that observed for proteins in the same expression system. Data for the MAD20 allele are presented in Fig. 1*B* ($\rho = 0.52$, $p < 0.0001$). Finally, levels of IgG Abs to the two other major MSP-1_{19} variants, E-KNG and Q-TSR expressed in yeast, correlated strongly with each other and with levels to the Wellcome/K1 and MAD20 alleles ($\rho = 0.85–0.89$, $p < 0.0001$). Age did not correlate with the prevalence or level of IgG or IgG subclass Abs to any of the four recombinant MSP-1_{19} proteins expressed in yeast. The example of IgG Abs to MAD20 allele of MSP-1_{19} is shown in Fig. 1*C*. Ab levels above and below the 75th percentile are separated by the dotted line in Fig. 1*C*.

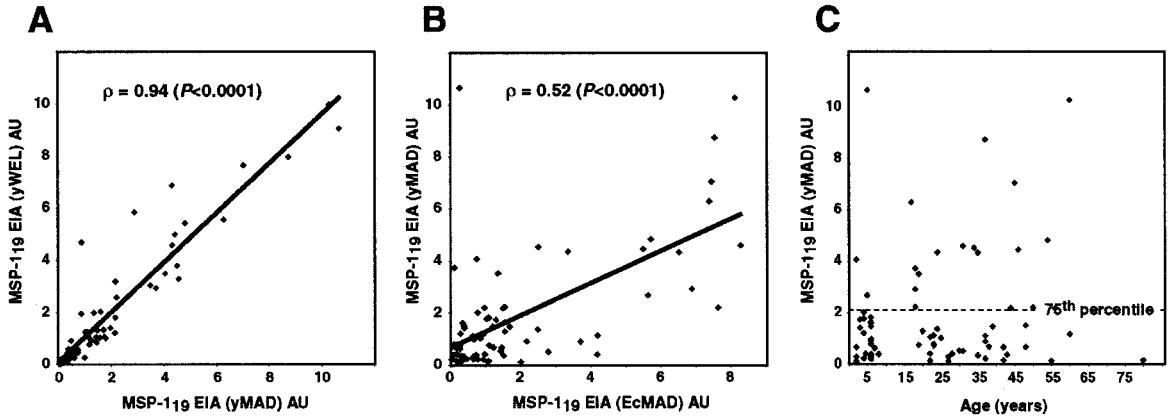

FIGURE 1. Pretreatment MSP-1_{19}-specific IgG levels in Kenyan plasma samples measured by serology using ELISA. *A*, Comparison of ELISA results, represented as arbitrary units (AU), obtained with yeast (*Saccharomyces*)-expressed MSP-1_{19} representing the MAD20 allele (yMAD) and Wellcome/K1 (yWEL) allele. The value ρ denotes the Spearman's correlation coefficient. *B*, Comparison of ELISA results for MSP-1_{19} (MAD20 allele) expressed in yeast and *E. coli* (EcMAD) is shown. *C*, Relationship of MSP-1_{19} IgG to yeast-expressed MAD20 allele to age is shown. The dashed line indicates the 75th percentile.

MSP-1$_{19}$-specific Abs dominate IIA activity

IIA were evaluated using the wild-type *P. falciparum* D10 and transgenic PcMEGF lines. The two lines are isogenic except for their MSP-1$_{19}$ domains (34). Plasma from the Kenyan subjects was considerably more effective at inhibiting invasion of wild-type D10 parasites than was the mutant D10 PcMEGF line (Fig. 2*A*). The median percentage of parasitemia for the D10 and D10-PcMEGF lines for all 76 Kenyan samples combined was 57.5% and 89.0% of nonimmune human sera. Controls in each assay included rabbit antisera specific for either *P. falciparum* MSP-1$_{19}$ or the antigenically distinct *P. chabaudi* MSP-1$_{19}$ domain that is expressed in the D10 PcMEGF line in place of the wild-type domain. These two monospecific sera differentially inhibited the D10 and D10 PcMEGF line as expected (34). The finding that a large proportion of the IIA activity in many samples from this Kenyan population is specific for MSP-1$_{19}$ is consistent with earlier observations in other malaria-exposed populations in Papua New Guinea and Africa (our unpublished observations).

The plasma samples were tested twice in independent IIA assays performed several months apart (assays 1 and 2). A strong correlation was observed for MSP-1$_{19}$-specific IIA values calculated from the two assays ($\rho = 0.86$, $p < 0.0001$; Fig. 2*B*). MSP-1$_{19}$-specific IIA did not correlate with IgG measured by ELISA (the example of yeast-expressed MAD20 allele is shown in Fig. 2*C*) or with age (Fig. 2*D*). Interestingly, the two individuals shown in Fig. 2*D* with the highest MSP-1$_{19}$-specific IIA levels (72 and 75% invasion-inhibition, above the dotted line) did not have IgG Abs to MSP-1$_{19}$ detected by ELISA.

MSP-1$_{19}$-specific IIA but not MSP-1$_{19}$ IgG Ab detected by serology correlates with delayed time to infection

Time to infection after administration of sulfadoxine-pyrimethamine was similar whether or not individuals had high levels of MAD20 MSP-1$_{19}$-specific IgG (above the 75th percentile) detected by ELISA (Fig. 3*A*). This lack of association was observed when age, gender, or pretreatment infection status were

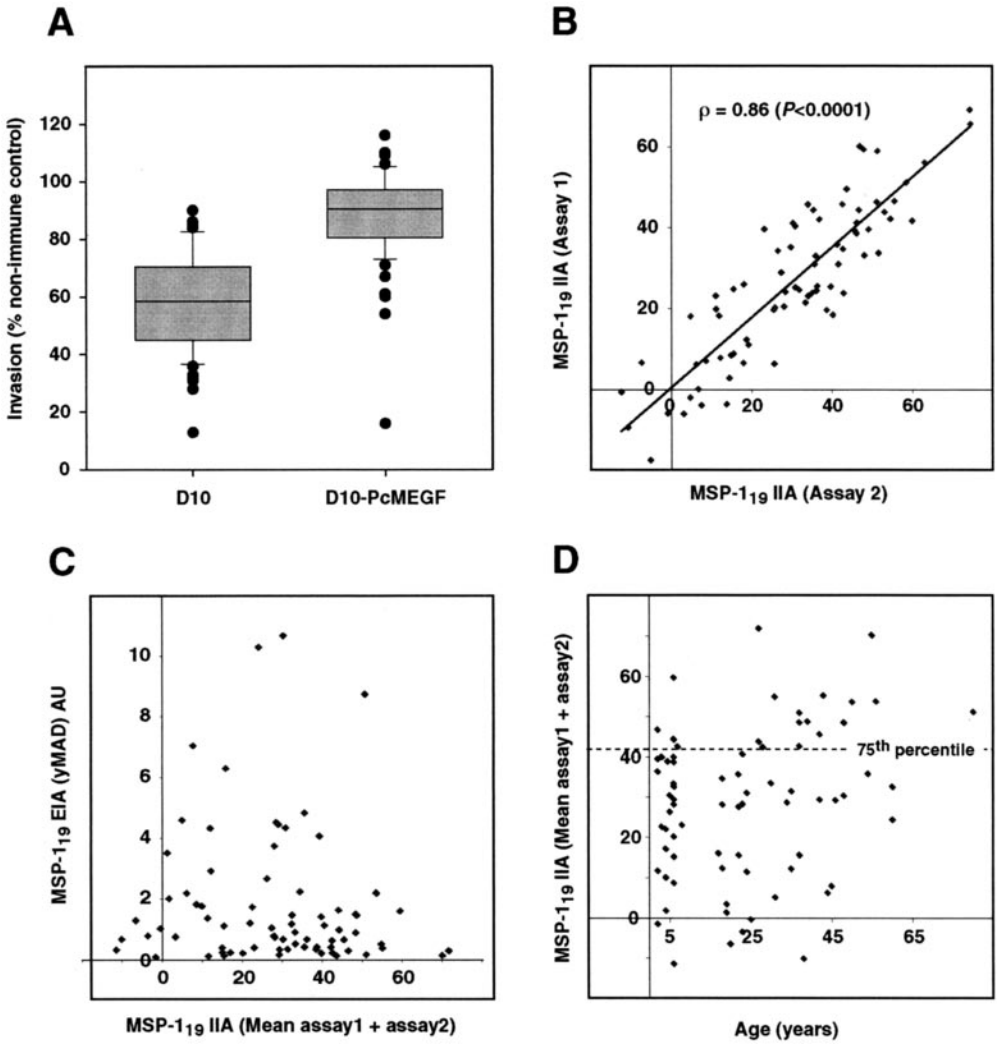

FIGURE 2. Invasion-inhibitory activity of plasma against wild-type and MSP-1$_{19}$ transgenic *P. falciparum* cell lines. *A*, Microscopy-based invasion-inhibition assays involving the detection of ring-stage D10 and D10 PcMEGF parasites after cultivation in the presence of individual plasma samples are shown. Invasion is expressed as a percentage of the invasion observed for parasites cultured in nonimmune control sera (human NIS) and the results are represented in a box plot. The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Error bars at the *top* and *bottom* of the box plot represent the 90th and 10th percentiles. Outlying points (●) have been included. *B*, Correlation between MSP-1$_{19}$-specific IIA levels calculated in two independent assays. MSP-1$_{19}$-specific IIA is calculated by subtracting the invasion rate of D10 from D10 PcMEGF for each individual plasma sample. *C*, Correlation between MSP-1$_{19}$ IgG detected by ELISA and mean MSP-1$_{19}$ IIA is shown. *D*, Comparison between age and MSP-1$_{19}$-specific IIA is represented. The dashed line indicates the 75th percentile, above which sera were said to have a high MSP-1$_{19}$ IIA (≥42.5%).

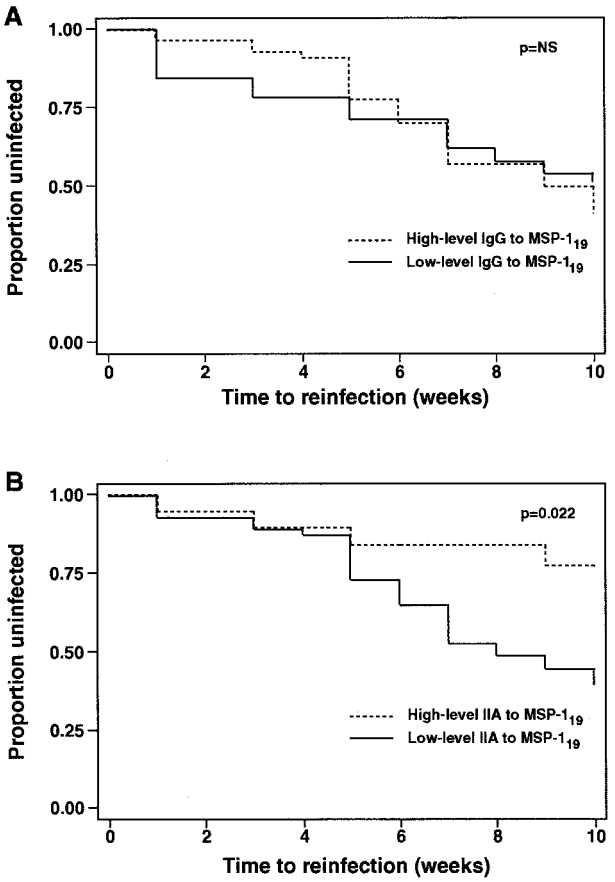

FIGURE 3. Relationship of MSP-1_{19} Abs with time to infection. *A*, Time to *P. falciparum* infection in individuals with high-level Abs to MSP-1_{19} measured by ELISA (above the 75th percentile in arbitrary units ≥2.18) as compared with low-level IgG Abs to PfMSP-1_{19} (below the 75th percentile in arbitrary units <2.18). *B*, Time to *P. falciparum* infection in individuals with high-level IIA activity to MSP-1_{19} (above the 75th percentile, IIA ≥42.5%) as compared with low-level IIA to MSP-1_{19} (below the 75th percentile, IIA <42.5%).

included as covariates in the analysis (adjusted hazard ratio approached 1 in all instances) and when ELISA was performed with *E. coli*-derived fusion proteins instead of the yeast-expressed Ags (data not shown). There was also no correlation between time to or risk of infection and total IgG, IgG1, and IgG3 Abs to MSP-1_{19} (e.g., the hazard ratio for IgG was 1.33; 95% confidence interval 0.62, 2.87). Median levels of IgG and IgG subclass Abs were similar among individuals who developed parasitemia vs those who did not (IgG, 0.90 vs 1.04 arbitrary units, IgG1 0.48 vs 0.32, and IgG3 0.37 vs 0.44; all *p* values not significant).

In contrast, persons with high-level MSP-1_{19}-specific IIA (above the 75th percentile) had a significantly prolonged time to infection compared with those with low-level (below the 75th percentile) MSP-1_{19}-specific IIA ($p = 0.022$, log-rank test, Fig. 3*B*). The median times to infection in the former and latter group were 9 and 7 wk, respectively. The reduced risk in persons with high-level MSP-1_{19}-specific invasion-inhibition values was significant after controlling for age, blood-stage *P. falciparum* infection before drug administration, and pre-existing anti-MSP-1_{19} Abs detected by ELISA with any of the recombinant MSP-1 proteins (hazard ratio 0.34, 95% confidence interval 0.12, 0.97). The median percentage of MSP-1_{19}-specific inhibition of invasion was higher in those who did not develop parasitemia but this difference did not reach statistical significance (33.0 vs 23.4%, $p = 0.2$).

No clear dose-response association for IIA was seen. The protective effect appeared limited to individuals in the highest quartile of MSP-1_{19}-specific IIA, with hazard ratios (95% confidence intervals) of 1, 1.03 (0.43, 2.48), 0.93 (0.38, 2.25), and 0.33 (0.11, 1.06) when comparing the first through fourth quartiles.

Discussion

Data presented in this study demonstrate that residents of a highland malaria mesoendemic area of Kenya have IgG Abs with functional invasion-inhibitory activity directed against the C-terminal MSP-1_{19}. These Abs accounted for most of the invasion-inhibitory activity detected, a finding consistent with previous observations in high transmission areas of Papua New Guinea (34). Importantly, persons with high-level MSP-1_{19}-specific IIA had substantially reduced risk (66%, 95% confidence interval 3–88%) of blood-stage infection in the 10 wk following administration of antimalarial drugs and clearance of any prior parasitemia. In contrast, IgG or IgG subclass Abs to recombinant MSP-1_{19} detected by serology did not correlate with invasion-inhibition activity or risk of infection. Overall, the data implicate an important protective role for Abs to MSP-1_{19} that prevent the functioning of MSP-1_{19} in the invasion of erythrocytes elicited in response to natural *P. falciparum* infection.

The lack of association of total IgG or IgG subclass Abs to MSP-1_{19} measured by ELISA with either invasion-inhibitory activity or protection against infection has several important implications. Firstly, the results demonstrate that MSP-1_{19} recombinant proteins expressed in various systems do not react identically with human Abs elicited by natural infection. Secondly, as shown by others (15, 25, 41), there is partial but not complete cross-reactivity of IgG and IgG subclass Abs with the four major variants of MSP-1_{19} defined by amino acid substitutions at positions 1644, 1691, 1700, and 1701, i.e., the E-TSR MAD20, Q-KNG Wellcome/K1, E-KNG, and Q-TSR alleles. These findings may be due to the possibility that the conformers/oligomers of the yeast- and *E. coli*-derived recombinant proteins differ from each other as well as native MSP-1. However, in prior studies in populations in malaria endemic areas of Africa, Abs to the *E. coli*-derived MSP-1_{19} recombinant protein were associated with protection from clinical malaria (26). In addition, Abs to the yeast-derived MSP-1_{19} recombinant protein were associated with protection from parasitemia and febrile illness (30). The *E. coli*- and yeast-derived MSP-1_{19} recombinant proteins used in the present study would therefore be expected to have conformations similar to these previously tested Ags. Thirdly, mAbs that prevent merozoite invasion of erythrocytes by inhibiting proteolytic processing of the 42-kDa to 19-kDa fragment of MSP-1 as well as those that block this inhibitory activity have been described (42, 43). Similar to the findings presented in this study, examination of sera from Nigerian children showed no correlation between anti-MSP-1_{19} Abs measured by serology and Abs that inhibited the processing of native MSP-1_{42} to MSP-1_{19} (44). Thus, although serologic assays presumably detect MSP-1_{19} Abs with multiple specificities (given the caveats previously mentioned), the MSP-1_{19}-specific IIA assay used in this study measures inhibitory activity of Abs targeting the region of MSP-1 containing the two essential EGF-like domains that function in the presence of the aforementioned blocking Abs. Measurement of MSP-1_{19}-specific IIA, a functional assay, would thus be expected to correlate more strongly with protection from infection than with anti-MSP-1_{19} Abs measured by ELISA even when the latter use optimally conformed MSP-1_{19} recombinant constructs. The present study demonstrates that in a naturally exposed population, the invasion of inhibition assay was in fact a superior predictor of protection from infection. Although we did

not determine the genotypes of MSP-1_{19} alleles in infected study subjects, prior studies have demonstrated extensive cross-reactivity of IIA to the variant MSP-1_{19} and MSP-1_{42} epitopes (15, 45), suggesting that the levels of IIA to other alleles possibly circulating in this area would be similar to the MAD-20 allele tested in this study.

From the perspective of immunogenicity, our results raise the possibility that the protective epitopes of the native protein may be poorly represented in at least some recombinant constructs of MSP-1. It will thus be of interest to determine whether MSP-1 constructs that have been recently been found to protect *Aotus* monkeys against blood-stage *P. falciparum* also induce high levels of anti-MSP-1_{19} Abs detectable by both ELISA and IIA (21, 46). The levels of anti-MSP-1_{19} Abs in the present study were similar to those seen in earlier studies (26, 28) and suggest that the high-level anti-MSP-1_{19} Abs detected by ELISA in vaccinated *Aotus* monkeys (46) differ in their specificities from those induced by natural infection of humans.

Studies of malaria-exposed populations in which time to infection after administration of antimalarial drugs or seasonal increases in transmission has been evaluated have generally been concerned with examination of immunity to Ags expressed by pre-erythrocytic *P. falciparum* (47, 48). Interpretation of this parasitologic endpoint is predicated on the assumption that immunity to pre-erythroyctic and liver-stage *P. falciparum* are the most important variables that determine time to development of blood-stage infection. A high degree of immunity to merozoites released from hepatic schizonts may however also result in delay of time to blood-stage infection. Indeed, our earlier studies in residents of the highlands of Kenya reported that T cell IL-10 responses to antigenic peptides of liver stage Ag-1 were weakly associated with time to infection, but found no correlation with T cell IFN-γ or Ab responses to this Ag (37). Studies of mice challenged with *P. yoelii* sporozoites indicate that immunization with the C-terminal fragment of *P. yoelii* MSP-1 leads to protection against blood stage infection by immunity directed against liver-stage parasites (49). Using transgenic *P. berghei* that express the *P. falciparum* MSP-1_{19} orthologue, we have observed that protection against blood stage challenge and peak parasitemia in mice rendered immune by repeated infection and drug cure correlates with MSP-1_{19}-specific invasion-inhibitory activity but not with the presence or level of Abs to MSP-1_{19} measured by serology (50). It was not possible in the present study to determine whether MSP-1_{19}-specific IIA correlates with peak parasitemia or symptoms of malaria because treatment with antimalarial drugs was mandated when asexual parasitemia first became detectable. The relatively low frequency of infection during the first 4 wk of the study likely reflects the prolonged effect of prior sulfadoxine-pyrimethamine treatment. Although the exact rates of sulfadoxine-pyrimethamine resistance in this highland area at the time the study was done (1997) are unknown, the eradication of infection at 2 wk in 82 of 84 individuals treated with sulfadoxine-pyrimethamine suggests that high-frequency sulfadoxine-pyrimethamine resistance did not exist at the time. Other potentially protective factors, such as bednet use and the presence of sickle cell trait or glucose-6-phosphate dehydrogenase deficiency, are unlikely to have affected risk of infection in this population: only 2 of the 76 study participants (2.7%) used bednets, and studies from a nearby highland area of Kenya demonstrated that frequencies of sickle cell trait and glucose-6-phosphate dehydrogenase deficiency are low in these populations (3% and 1%, respectively) (51).

Our findings provide evidence that MSP-1_{19}-specific invasion-inhibitory activity as measured by an in vitro assay that uses transgenic *P. falciparum* is an immune correlate of protection against blood-stage *P. falciparum*. The study was conducted in an area where transmission of malaria is episodic and characterized by some as "mesoendemic" (7). Further investigations will be required to establish whether a similar relationship exists in endemic areas where transmission is stable and higher than in the Kenya highlands, such as in infants in the lowlands of western Kenya who experience higher levels of asexual parasitemia (29). A dose-response relationship, which was not observed in the present study, might be seen in a population with higher IIA levels. Clinical surveillance conducted in a setting where treatment is indicated only for symptomatic malaria will also be necessary to determine whether MSP-1_{19}-specific invasion-inhibitory activity is associated with a decrease in the peak level and incidence-density of parasitemia as well as malaria-attributable fever. Finally, it will be important to examine a larger number of individuals to have sufficient statistical power to determine whether lower levels of MSP-1_{19}-specific invasion-inhibitory activity correlate with protection against malaria.

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