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Short Title: Antibodies to MSP-1p19 and protection against malaria

Antibodies to the conserved C-terminal domain of the *Plasmodium falciparum* merozoite surface protein-1 and to the merozoite extract and their relationship with in vitro inhibitory antibodies and protection against clinical malaria in a Senegalese village

Ronald Perraut[1*], Laurence Marrama[2], Babacar Diouf[1], Cheikh Sokhna[3], Adama Tall[2], Pierre Nabeth[2], Jean-François Trape[3], Shirley Longacre[4] & Odile Mercereau-Puijalon[4]

[1]Unité d'Immunologie, Institut Pasteur de Dakar, Sénégal

[2]Unité d'Epidémiologie, Institut Pasteur de Dakar, Sénégal

[3]Laboratoire de Paludologie/ Zoologie Médicale, IRD, Dakar, Sénégal

[4]Unité d'Immunologie Moléculaire des Parasites, CNRS URA 2581, Institut Pasteur, Paris, France

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The project protocol and study design were explained to the assembled villagers, and informed consent was obtained individually from all participants or their parents or guardians. The protocol was approved by the Ethics Committee of the Ministry of Health of Senegal.
The authors do not have any commercial or other association that might pose a potential conflict of interest[1]

*Address for correspondence: Dr Ronald PERRAUT, Unité d'Immunologie, Institut Pasteur de Dakar, 36 Av. Pasteur, BP-220, DAKAR, SENEGAL
Phone: 221 839 92 45   FAX: 221 839 92 10 - Electronic Mail: perraut@pasteur.sn

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ABSTRACT

Antibodies that react with *Plasmodium* merozoite antigens are thought to play a key role in clinical immunity to malaria. Antibodies specific to the conserved, structurally constrained C-terminal region of the *Plasmodium falciparum* major merozoite surface protein 1 (MSP-1p19) have been shown to inhibit merozoite invasion of erythrocytes *in vitro*, and more importantly, are associated with protection against clinical episodes of malaria. However, it is unclear if these observations relate specifically to MSP-1p19 or whether they apply more generally to all merozoite antigens. To answer this question, we report here a prospective serological study conducted in the mesoendemic Senegalese village of Ndiop, in which 205 individuals were enrolled before the onset of the 2000 rainy season, in an active clinical survey during the following five months. The relationships between antibody responses to either a recombinant baculovirus MSP-1p19 antigen or a whole merozoite extract, the serum capacity to inhibit *in vitro* parasite growth or erythrocyte invasion, and their correlation with protection against clinical malaria were compared. The levels of antibodies to MSP-1p19 or the merozoite extract were age-associated, but the capacity to inhibit parasite growth or invasion *in vitro* was not. Merozoite extract seropositivity was associated with growth inhibition, while invasion inhibition was associated with elevated IgG levels to MSP-1p19. Most importantly, high levels of anti-MSP-1p19 IgG were associated with a reduced occurrence of malaria attacks in an age-adjusted multivariate analysis. These results strongly support further MSP-1p19-based vaccine development.

Keywords: *Plasmodium falciparum*, IgG response, MSP-1p19, protection, inhibition assays

INTRODUCTION

Progress towards malaria vaccine development requires a better understanding of the immune mechanisms contributing to the natural protection of individuals living in endemic areas. Sero-epidemiologic studies have found associations between antibody responses to specific *Plasmodium falciparum* antigens and protection against clinical disease. Several antibody-mediated mechanisms operate to restrict parasite multiplication, including inhibition of erythrocyte invasion [1, 2], inhibition of merozoite release [3], intracellular parasite killing [4] and destruction of infected red blood cells [5, 6]. In the context of vaccine development, antigens showing restricted diversity are of particular interest. One of the most conserved antigens of these is the C-terminus of merozoite surface protein-1 (MSP-1) [7, 8]. MSP-1 is produced as a high molecular mass precursor that is membrane-anchored via a glycosyl phosphatidyl inositol moiety. It undergoes two successive proteolytic processing steps during merozoite maturation [9], leading to a final conserved C-terminal membrane anchored moiety called PfMSP-1p19, which consists of two structurally constrained EGF-like domains [10, 11]. The response to PfMSP-1p19 is complex, including antibodies that inhibit erythrocyte invasion by merozoites, as well as antibodies that compete with the inhibitory antibodies [12, 13]. Inhibition of erythrocyte invasion has been associated with the presence of antibodies blocking the final proteolytic step that produces PfMSP-1p19 [13, 14], and with protection in a murine model using PfMSP-1p19 transgenic *P. berghei* parasites [15].

The antibody response to PfMSP-1p19 has been positively correlated with clinical immunity in children and adults in some settings [16-21], but not others [22, 23]. The relevance of these associations has rarely been evaluated vis-à-vis their putative underlying function, namely inhibition of merozoite invasion. Recently, O'Donnell et al reported that antibodies to PfMSP-1p19 are major contributors of the invasion inhibitory activity present in

the serum of immune adults [24]. However, it is not clear how this relates to protection from clinical disease, or to other potentially protective mechanisms targeting other merozoite antigens. The aim of this study was to document the relationship of the specific anti-PfMSP-1p19 antibody response to the more general anti-merozoite antibody response, and to investigate their association with both growth and/or invasion inhibition *in vitro*, and protection against clinical malaria in an endemic population. We conducted a prospective serological study in Ndiop, a Senegalese village with moderate and seasonal transmission [25], where a longitudinal prospective study has been conducted for more than ten years [26, 27]. The study included 205 plasma samples collected just before the rainy season, followed by monitoring of clinical malaria episodes throughout the subsequent transmission season by active daily case surveillance. The anti-merozoite antibodies were evaluated using a crude merozoite extract, prepared from the FCR3 reference strain. Importantly, a baculovirus expressed, secreted recombinant protein was used to evaluate. the anti-PfMSP-1p19 antibody response, since it involves reduction-sensitive epitopes [2, 28, 29]. This system was shown to ensure homogeneous folding of the structurally constrained recombinant product, whose crystral structure revealed proper folding of both EGF domains [11]. In addition, we evaluated individual sera with regard to two *in vitro* functional assays, inhibition of parasite growth and inhibition of erythrocyte invasion, and their relationship with the number of clinical episodes during the subsequent transmission season.

MATERIALS AND METHODS

Study area, study design and procedures

The study was conducted in Ndiop, Senegal, a village with moderate seasonal transmission [25, 26] after approval by the ad hoc Ethics Committee of the Ministry of

Health. The protocol of follow-up was explained to the assembled village population and informed consent was obtained from the villagers. Any individual could withdraw from the study and the follow-up procedure at any time.

Briefly, in July 2000, 205 healthy villagers (108M/97F) aged 3.6 to 75 years (mean age 23.9 years) were enrolled for a cross-sectional prospective follow-up. The cohort comprised 36, 4 and 165 individuals with hemoglobin AS, AC and AA, respectively. Sampling of all villagers took place between July 17th and August 1st 2000. Thirty-seven villagers had a microscopically positive peripheral parasitemia at the time of blood sampling (range 0.5 - 80 trophozoites per 100 leucocytes). The detection threshold of the microscopic examination is 1 parasite per microliter in our hands [30]. None of the villagers recruited had used anti-malarials for at least 4 weeks before blood withdrawal. After venous puncture, plasma and red blood cells were separated by centrifugation and stored at -20°C.

Active clinical surveillance was carried out over a five-month period encompassing the malaria transmission season, from August 1st to December 31st 2000 as described [26, 30, 31]. The protocol included the notification of all febrile episodes and controlled use of anti-malarial drugs by the medical staff. Each villager was visited daily at home for clinical surveillance, and blood films were made in case of fever, and read extemporaneously. A malaria attack was operationally defined as an association of symptoms suggesting malaria with parasitemia >30 trophozoites/100 leukocytes. An anti-malarial drug cure was administered by the medical staff following each positive diagnosis for malaria. A total of 278 clinical episodes were treated with anti-malarials. Blood films were subsequently read a second time for proper quantification of parasitaemia.

In parallel, the entomological inoculation rate (EIR) was monitored weekly as described [25]. Cumulative EIR for the entire transmission season was estimated as 50.75

infective bites per individual from the end of July 2000 to mid October 2000. No transmission was recorded in November and December 2000 in the village.

Antigens and ELISA procedure

The merozoite extract was prepared from synchronous FCR3 parasites cultivated on O^+ erythrocytes and 10% human serum in candle jars as described [31]. Briefly, the merozoite extract was prepared from synchronous FCR3 parasites raised in candle jars in O^+ erythrocytes, 10% human serum. Merozoites, collected after stepwise centrifugation at 2,000 rpm and 4,000 rpm, were washed three times in sterile phosphate-buffered saline (PBS), counted and frozen.

The extract was used to coat MaxiSorp® plates (Nunc, Roskilde, Denmark) at 10 $\mu g.mL^{-1}$ [32]. MSP-1p19 (Palo Alto allele) was produced in *Spodoptera frugiperda* (Sf9) or *Trichoplusia ni* (High Five, Invitrogen) insect cells infected with the recombinant baculovirus, purified by metalloaffinity chromatography [33], and used to coat Immulon-4 plates at a concentration of 0.5 $\mu g\ mL^{-1}$, after dilution in sterile PBS.

Assays for the determination of IgG responses were performed by ELISA as described [31, 32, 34]. Plasma samples were tested in duplicate at a dilution of 1/200. A negative control (a pool of European and/or African non-immune sera) and a positive control (a pool of 25 sera from clinically immune adults living in Dielmo and Ndiop) were included in each assay to ensure comparability between the plates. Results were expressed as OD ratios = OD sample / OD naive serum pool [31, 34]. Sera from individuals with an OD ratio > 2, which exceeds the signal of naive controls +2SD (OD ratio= 1.9) were considered to be sero-positive.

Growth Inhibition and inhibition of invasion assays

The growth inhibition assay (GIA) was done using sorbitol synchronised cultures. Young trophozoite stage parasites were adjusted to 0.4 % parasitemia at 1,5% hematocrit in a 96 well tray. Plasma, tested in triplicate at a 1/10 dilution in RPMI 0.5% Albumax®, were incubated for 24 h at 37°C in candle jars. Medium was removed and 25 µL [^{3}H]hypoxanthine was added (1 µCi/well), and further incubated for 24 hr. Plates were then frozen and thawed to lyse infected RBCs. Samples were transferred to a glass fiber filters and quantified using a β scintillation counter (Wallac, Trilux®). Inhibition of invasion was carried out essentially as described by O'Donnell et al [24], except that the FCR3 strain was used [35].

Randomly chosen sera (N=94) from gender and age stratified groups (0 - <15y, 15-29 and >30years) were tested in parallel in both assays. This included 51 Males and 43 Females; the mean age was 25.7 years (range 3.6 to 75 y). Negative controls consisted of medium alone a commercial pool of non-immune European donors (Valbiotech, France) and a pool of 20 African non-immune sera. A pool of 25 immune sera was used as a positive control [32, 35]. The % inhibition was calculated as ([mean cpm $_{\text{negative control}}$ - mean cpm $_{\text{sample}}$] / mean cpm $_{\text{negative control}}$) x100.

Statistical analysis

Comparisons of antibody levels and/or growth or invasion inhibition in different groups were done by means of the Wilcoxon signed rank test and the Spearman rank correlation test for non-normally distributed paired data. *P* values <0.05 were considered significant.

A Poisson regression model was used to analyze the relationship between antibody response(s) and incidence of malaria attacks during the follow-up period. For the analysis, a *P. falciparum* malaria attack was defined as presence of fever or symptoms suggesting

malaria associated with >30 *P. falciparum* trophozoites/100 leukocytes, as ascertained by re-examination of all slides by a highly experienced microscopist. This showed that 31 of 278 antimalarial treatments administered did not fullfill the strict definition of "*P. falciparum* malaria attack". There were 2 clinical malaria with *P. malariae*, 6 with *P. ovale* and 23 with a *<30 P. falciparum* trophozoite/ 100 leukocytes. For each villager, the follow-up time was calculated as the number of days actually spent in the village during the five months of follow-up. Seven villagers, who were out of the village for more than 30 days during the follow-up period, were excluded from the malaria incidence analysis. Thus, the analysis included 192 individuals, who experienced a total of 278 clinical malaria episodes during the follow-up period. The incidence of clinical malaria episodes (per 1000 person-days) was 29 [24-35] for the 0-14 y age group (n = 77), 9 [6-13] for the 15-29 y age group (n = 58) and 2 [1-4] for the ≥ 30 y age group (n = 57) (Wald test, $P < 0.001$). Malaria attacks were considered independent if separated by more than 15 days. The follow-up time was adjusted for individuals who experienced malaria attacks, by excluding from the days at risk a period of 15 days after the diagnosed malaria attack. It was also adjusted for the individuals who received an anti-malarial treatment without fulfilling the strict malaria attack definition, by excluding a period of 8 to 15 days after the first day of treatment (8, 10 and 15 days for quinine, chloroquine and sulfadoxine-pyrimethamine, respectively).

Optimal age stratification was based on the age distribution of the parasitologic and clinical data available for this setting based on the 10-year longitudinal follow up of the entire population [26, 30]. The ages of 15 years and 30 years were used as cut-offs [31]. First-level interactions between variables were checked and included in the model when significant. The antibody level stratification was determined using Aikake's information criterion.

Statistical analyses were performed with Egret 3.01® (Cytel) and Statview 5.0® (SAS Institute) software.

RESULTS

Prevalence and quantification of antibodies to the merozoite extract and PfMSP-1p19

The prevalence sera reacting with the merozoite extract and the PfMSP-1p19 antigen was very high, with 96% and 79% of villagers scored as positive, respectively. The mean OD ratios to the merozoite extract and PfMSP-1p19 were 3.1± 0.6 (corresponding to OD values of 1.1 ± 0.33) and 8.1± 6.1 (OD values of 0.97 ± 0.79), respectively. For both responses, the mean and median OD ratios coincided. The IgG responses to the merozoite extract and to PfMSP-1p19 were positively correlated (Rho = 0.36; $P < 10^{-4}$), confirming previous results [31] and both were positively associated with age ($P < 10^{-4}$; Rho = 0.32 and 0.37, for the merozoite extract and MSP-1p19, respectively) (Figure 1). They were not associated with gender, hemoglobin type, or detectable parasitemia on the day of blood sampling.

Inhibitory activity in functional assays and relationship with ELISA responses

A subset of 94 plasma with a representative age distribution was analyzed for the presence of antibodies affecting parasite multiplication using two functional assays, a growth inhibition assay (GIA) and an erythrocyte invasion inhibition assay (EIIA). Interestingly, for both assays the antibody response showed a close to normal distribution (Shapiro-Wilk, *P*=0.1 for both) (Figure 2a and 2b for GIA and EIIA, respectively). A large proportion of individuals had inhibitory antibodies (i e showed >10% inhibition in the assay). All plasma tested presented inhibitory activity in the GIA, ranging from 26% to 81% inhibition. The mean value for GIA was quite high 55.7% ± 13%. For the EIIA, only 2 of 94 individuals displayed >10% inhibition. The mean value for EIIA was 39.5 % ±15.2% (range 10% - 75%).

The inhibitory responses in the GIA and EIIA were positively correlated (*P*<10^{-4}, Rho=0.42), but importantly for both assays, there was no association of inhibitory activity with age. Interestingly, the ELISA response to the merozoite extract was positively correlated with inhibitory activity in the GIA (Spearman rank test, *P*=0,004, Rho=0.30), but not in the EIIA. A dual-level stratification of the antibody response to the merozoite extract showed a higher growth inhibition capacity in the GIA (*P*=0.02) for individuals with ≥ 2.5 IgG ratios (Figure 3a).

The unstratified antibody response to MSP-1p19 showed no correlation with inhibitory activity in the GIA or the EIIA. However, a dual-level stratification showed that individuals with a high level of MSP-1p19 antibody (namely OD ratio ≥ 7) had a significantly higher inhibitory activity in the EIIA (Figure 3b ; *P*=0.01), but not in the GIA (Figure 3a). Thus, the ELISA responses to the merozoite extract and to MSP-1p19 were associated with distinct *in vitro* functional assays.

Relationship between antibody responses and the number of clinical episodes

The relationship between the dichotomized antibody response and the incidence of clinical malaria episodes during the 5-month follow-up was analyzed using an age-adjusted Poisson regression model. The inhibitory activity in GIA or EIIA was unrelated to protection against clinical malaria during the subsequent 5 months. Likewise, the unstratified and stratified IgG response to the merozoite extract was not significantly associated with the incidence of clinical malaria (*P* =0.97). In contrast, there was a significant association of the stratified anti-MSP-1p19 response (OD ratio >7 vs. ≤ 7) with a reduced incidence of clinical malaria episodes (incidence rate ratio (IRR) = 0.77 [0.60-0.99], *P* =0.047).

A multivariate analysis showed that the variables significantly associated with the incidence of clinical malaria were: i) age (15-30 y. vs. > 30y.: IRR1 = 4.0; <15y. vs. > 30y.:

IRR2 = 12.7, *P*<0.001 for both), ii) hemoglobin characteristics (HbAS vs. others: IRR=0.55, *P*=0.001), iii) positive parasitemia at enrolment (IRR=0.69, *P*=0.02) and iv) the IgG response to MSP1 (≥ 7 OD ratio vs. <7: IRR=0.73 [0.55-0.96], *P*=0.03).

A striking characteristic of the relationship of the anti-MSP-1p19 IgG response with protection is illustrated in Figure 4. The cumulative incidence of clinical malaria episodes was quite distinct in the three age-groups considered. The incidence of clinical malaria in the >30y age group was low, whatever the level of anti-MSP1-p19 IgG, it was 2-3 fold higher in the 15-29 y age group and also essentially unrelated to the anti-MSP-1p19 response. However, there was a marked dichotomy in the younger age group, which accounted for 73% of the clinical attacks recorded during the 5-month follow-up. The children with a relatively lower level of anti-MSP-1p19 IgG experienced a much higher incidence of clinical malaria than those of the same age with relatively higher antibody levels to MSP-1p19. This dichotomy was particularly marked in the first month of the survey, but remained throughout the 5-month period.

DISCUSSION

A high percentage of the endemic population sera tested here were reactive with the merozoite extract and/or baculovirus recombinant PfMSP-1p19, both by ELISA and in the functional assays. Since no infected mosquito was captured in Ndiop from the end of September 1999 to the end July 2000, this denotes persistence of substantial levels of specific (or cross-reacting) antibodies over 10 months of highly reduced or interrupted transmission. Whether this reflects continuous antigenic stimulation resulting from chronic, low density parasite carriage or slow decay of strong responses triggered or boosted during the transmission season is unclear. Short-lived antibody responses to MSP-1 have been reported in humans [1, 22].

The magnitude of antibody responses relating to the four parameters investigated here is remarkable in view of the inherent limitation of the tests due to parasite polymorphism. Previous molecular epidemiology studies carried out in this setting has indeed indicated that allelic diversity is quite large [36-39]. Since the merozoite extract derives from the FCR3 line, which was also used for the inhibition assays, only a single allelic type for the various merozoite polymorphic antigens such as MSP1-4, AMA1, etc. is tested. Thus, the merozoite extract ELISA and the inhibition assays measure response to conserved antigens, and to an unknown fraction of the overall variable antigenic repertoire of the local parasite population. Allelic polymorphism is less of an issue for MSP-1p19, which is highly conserved. Indeed, MSP-1p19 sequences derived from 50 of 52 isolates collected in Ndiop and the near-by village of Dielmo, corresponded to two alleles differing at a single dimorphic position (E-KNG and Q-KNG), the former of which corresponds to the Palo Alto allele used in this study (S. Rosario et al, unpublished data). Previous work has indicated that, as expected with an antigen as conserved as MSP1-p19, there is substantial cross-reactivity between allelic forms by antibodies from malaria–exposed individuals [28].

Antibodies to MSP-1p19 and to the merozoite extract were positively correlated, and both antibody responses were age-related, confirming previous observations made in this setting three years earlier, also just before the onset of the malaria transmission season [31]. An age–related IgG response to MSP-1p19 has been documented in many endemic areas [18, 20, 21, 28, 40]. Depending on the study site, the prevalence of antibodies varies from 28 to 77 % [20, 21, 23, 28, 40, 41]. This cannot be attributed to variable intensity of transmission and probably reflects sub-optimal evaluation of the serological response using recombinant antigens produced in expression systems than do not ensure the homogeneous folding of conformation constrained epitopes provided by the baculovirus expression system [2, 33, 42].

Interestingly, neither growth nor invasion inhibitory activities were age-related, although sera from all villagers showed some growth inhibitory activity, and a very large proportion had invasion inhibitory antibodies. As anti-malarial use in the village is strictly monitored, invasion or growth inhibition due to the presence of these drugs in the plasma can be excluded. Thus, the inhibition activities can be attributed to specific antibodies, an interpretation that is supported by their correlation with specific ELISA responses. Growth inhibition was associated with the presence of antibodies reacting with the merozoite extract, whereas erythrocyte invasion inhibition was related only to high levels of MSP-1p19 specific antibodies. This suggests that GIA and EIIA measure independent responses, probably involving distinct antigen/antibody interactions. The association of anti-MSP-1p19 antibodies with the invasion inhibitory response is consistent with the correlation of higher level of anti-MSP-1p19 IgG1 antibody with lower parasite density in Kenyan children [19] and with the inhibitory activity of sera from malaria-exposed immune adults on MSP-1p19-transgenic lines [24]. We show here that this activity is not age-dependent, and is observed only in individuals who have high levels of antibody specific for MSP-1p19. Thus, it is not necessarily restricted to "immune adults". The association of invasion inhibitory activity with the response to MSP-1p19, rather than to the merozoite extract, is consistent with competing "blocking antibodies" reacting with MSP-1 sequences outside of MSP-1p19 [13]. An alternative explanation is that the response to MSP-1p19 is masked by responses to other merozoite antigens within the merozoite extract. Further work is needed to explore these possibilities.

Protection against clinical malaria was associated with a single specific immune signature, namely the presence of high antibody levels to MSP-1p19. In a similar prospective study conducted in Ndiop in 1997, a reduced incidence of clinical malaria was associated with the response to the merozoite extract (IRR = 0.804) but the association with the response to MSP-1p19 did not reach statistical significance, due to an age-related confounding effect

[31]. We attribute this discrepancy to the larger sample size of the present study (205 individuals vs. 110 subjects in the 1997 study). The data of the present study confirm the association of high levels of anti-MSP-1p19 IgG levels with protection against clinical malaria in Gambian children [21]. Interestingly, in Gambian children as in the Ndiop villagers, there was no association with the frequency of responders to MSP-1p19, consistent with observations in other settings [19, 21, 22].

The association of protection against clinical malaria with high MSP-1p19 IgG levels but not invasion inhibitory antibodies is in apparent conflict with the conclusions drawn from a newly developed mouse model of infection with transgenic *P. berghei* parasites expressing *P. falciparum* MSP-1p19 [15]. In mice immunized by infection and cure with transgenic parasites, protection following subsequent blood stage challenge was not associated with IgG levels to *P. falciparum* MSP-1p19, but was correlated with the levels of antibody inhibiting *P. falciparum* erythrocyte invasion *in vitro* [15]. However, "protection" in this mouse model refers to prevention of lethal hyperparasitemia, with reduced peak parasitemias and reduced clearance time. This differs substantially from the definition of protection against clinical malaria in humans. Indeed, the peak parasitemia observed in the "protected" mice (14.8%) would qualify the infection as hyperparasitemia in human malaria, where "protection" refers to control of peripheral parasitemia below 0.02% in the absence of clinical symptoms. In addition, homologous challenge is very unlikely in a mesoendemic setting such as Ndiop. Thus, the mechanisms implicated in protection in the mouse model and in endemic areas may not totally overlap.

This prospective survey allowed to investigate correlations of specific serological responses with protection against clinical malaria during the subsequent transmission season. The observed correlation of protection with antibody levels at recruitment obviously does not preclude contribution of additional immune responses to protection, including through rapid

boosting upon parasite exposure of anti-merozoite responses that were sub-liminal at the onset of transmission, or through mounting of novel responses directed against the variant red blood cell surface antigens [43]. The age-independent, hemoglobin-type-independent, association of high anti-MSP-1p19 IgG levels with a reduced incidence of clinical episodes argues strongly in favor of further vaccine development based on this antigen. Our data are consistent with the interpretation that high levels of antibodies to MSP-1p19 contribute to clinical protection, possibly by inhibiting merozoite invasion of erythrocytes. Since conformation-dependent antibodies to MSP-1p19 are mainly cytophilic [18, 34], they could also be involved in other immune mechanisms, such as monocyte-mediated antibody-dependent parasite killing [4].

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LEGENDS OF FIGURES

Figure 1: Distribution of the IgG response (expressed as OD ratio) to the merozoite extract (**a**) and to MSP-1p19 (**b**) by age and relationship of the responses to merozoite vs to MSP-1p19 (**c**).

Figure 2: Distribution of the inhibitory antibody responses in GIA (**a**) and EIIA (**b**).

The mean value-centered histogram plots the number of observations within ± 1 to 4 Standard Deviation from the mean value. For the GIA, the mean inhibition value was 55.7% ± 13. For the EIIA**,** the mean inhibition value was 39.5% ± 15.2.

Figure 3: Inhibitory antibody responses by dichotomized antibody responses against MSP-1p19 and merozoite extract.

GIA (**a**) and EIIA (**b**) inhibitory levels are shown for dichotomized IgG responses against MSP-1p19 : OD ratio < 7 (light grey , n=43, mean age=19.8y; 3.6-72y) or ≥7 (dark grey, n=51, mean age=30.8y; 5.4-75y), and against the merozoite extract : OD ratio < 2.5 (dashed light grey, n=16, mean age=17.5y; 5.4-51y) or ≥ 2.5 (dashed dark grey, n=78, mean age=27.4; 3.6-75y)**.** The asterisks indicate significantly different levels for: -i) growth inhibition assay and anti-merozoite response (*P*=0.02); -ii) reinvasion inhibition assay and anti-MSP-1p19 response (*P*=0.01).

Figure 4: Relationship between the incidence of clinical malaria attacks and the IgG responses against MSP-1p19 in different age-groups

The calculated cumulated incidence of clinical accesses is plotted by dichotomized IgG levels to MSP-1p19 < 7 (closed symbols) vs ≥ 7 (dashed lines, open symbols) in three age-groups as indicated. The upper right histogram represents the number of clinical malaria episodes recorded in the 3 age groups during the 5 mo daily clinical follow up.

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Figure 1

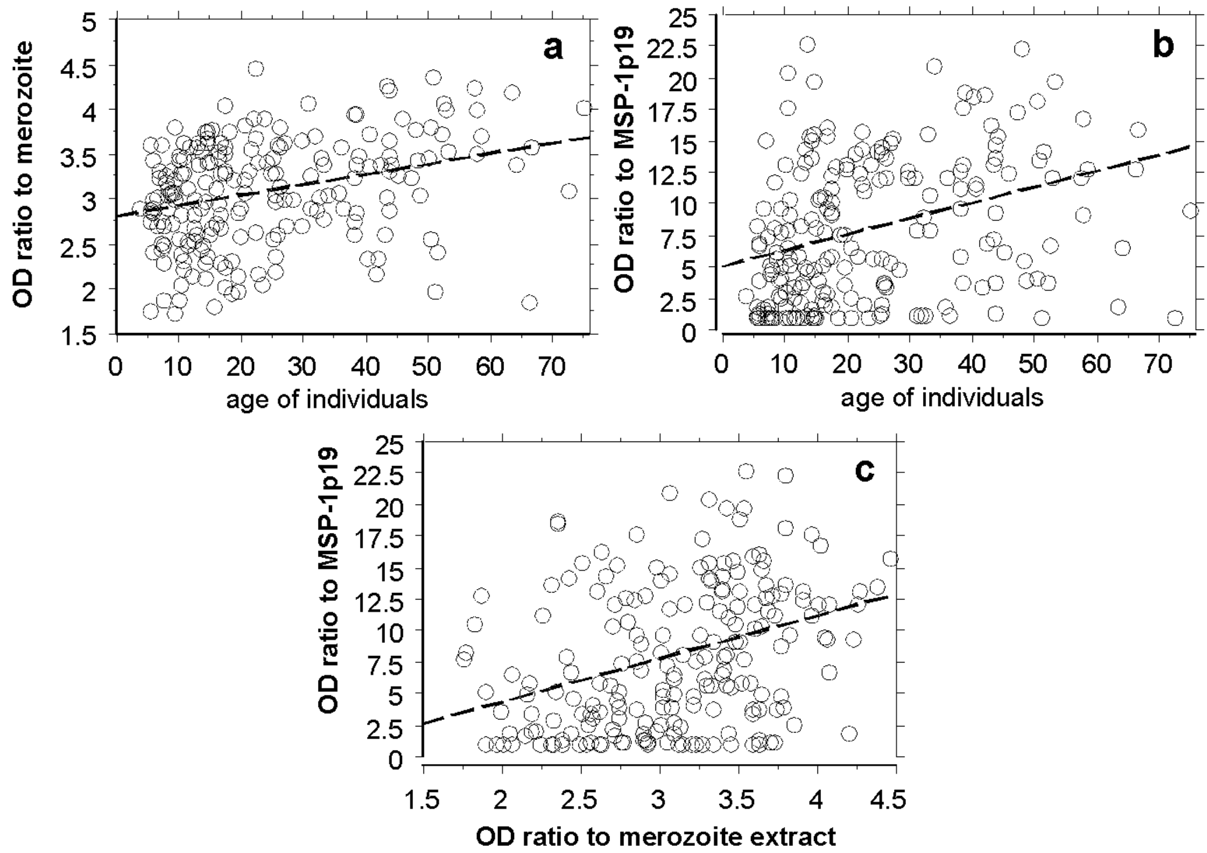

Figure 2

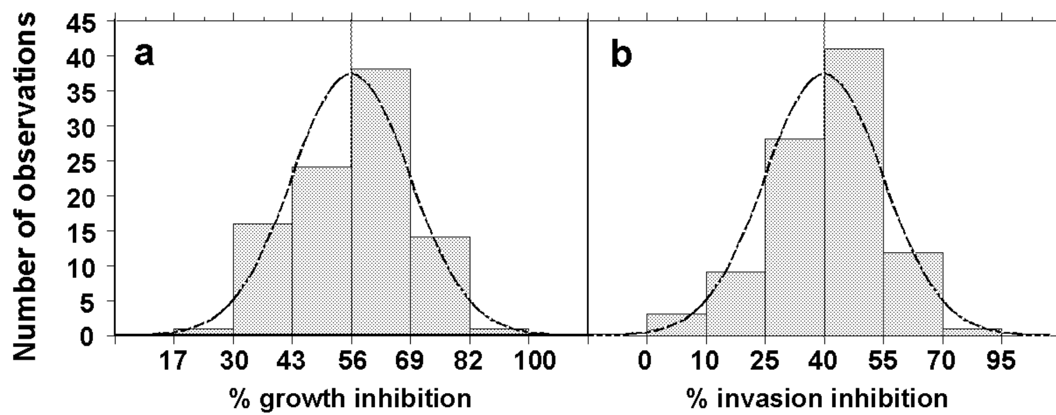

Figure 3

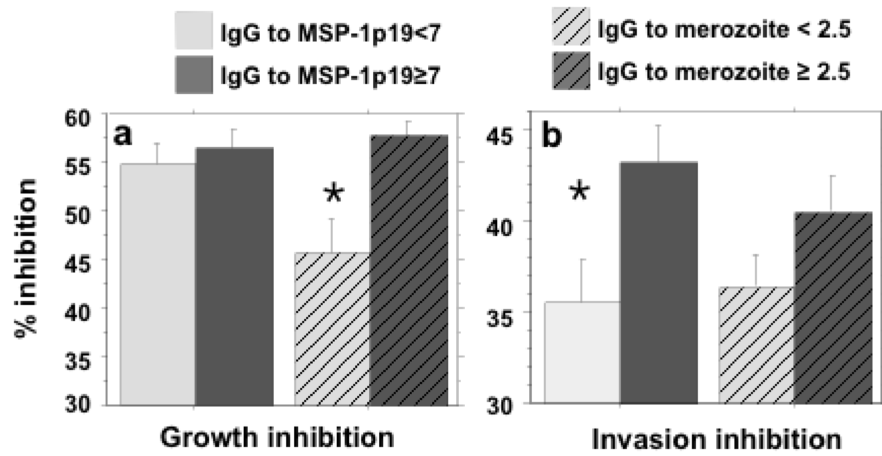

Figure 4