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HOST FACTORS AFFECTING THE DELAY OF REAPPEARANCE OF *PLASMODIUM FALCIPARUM* AFTER RADICAL TREATMENT AMONG A SEMI-IMMUNE POPULATION EXPOSED TO INTENSE PERENNIAL TRANSMISSION

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Abstract. To investigate host factors affecting the delay of reappearance of malaria parasites after radical treatment, a study was underdaken in Dielmo, Senegal, an area of intense perennial malaria transmission. A 7-day course of quinine was administered to 173 asymptomatic persons from 1 to 85 years of age and reappearance of malaria parasites in the peripheral blood was monitored weekly for 14 weeks. Additional thick blood films were made in case of fever as part of a daily clinical surveillance. The median times before reappearance of *Plasmodium falciparum* were 22, 39, and 53 days among persons 1–6, 7–14, and ≥ 15 years of age, respectively ($P < 0.0001$). Multivariate analysis indicated that the daily rate of reappearance of *P. falciparum* was 2.2 (95% confidence interval [CI] = 1.2–4.5) times lower in sickle cell trait carriers than in AA individuals, and 1.5 (95% CI = 1.1–2.1) times lower in bed nets users than in non-users. The risk ratio for the daily risk of reappearance was significantly related to the level of parasitemia before treatment. No influence of glucose-6-phosphate dehydrogenase deficiency, HLA-B53, and DR13 were observed. Findings show that monitoring during a few weeks the reappearance of malaria parasites after treatment among a small cohort of individuals naturally exposed to malaria is relevant for investigating host resistance factors. This suggest that small, low-cost, field trials may be very informative on the potential of new malaria vaccine candidates.

Recent studies in highly malarious areas have shown an interest in assessing the delay of reappearance of malaria parasites in the blood after treatment to investigate the immune defense mechanisms operating at the pre-erythrocytic level,[1,2] or to address the relationships among a continuum of sporozoite dose, duration of exposure, and recurrent parasitemia.[3–6] Such studies may also provide useful information for the design of malaria vaccine trials among communities naturally exposed to malaria. The comparison of the incidence of new *Plasmodium falciparum* infections among vaccine and placebo recipients after the administration of schizonticidal drugs has been recommended by the World Health Organization to test in the field the efficacy of malaria vaccines directed against pre-erythrocytic and asexual blood stages of *P. falciparum*.[7] However, few baseline data from communities living in highly endemic areas are available, since most studies measuring the delay of reappearance of malaria parasites in the blood following treatment were aimed to investigate drug resistance without exploring other factors that could affect this delay. Here we explore relationships between resistance to reinfection and a series of host factors among a Senegalese community naturally exposed to intense perennial malaria transmission.

MATERIALS AND METHODS

The study took place in the village of Dielmo, Senegal, with 250 inhabitants, an area of intense and perennial malaria transmission where the entire population was involved in a prospective study of natural malaria infection and the mechanisms of protective immunity, which is described in details elsewhere.[8,9] In January and in August 1992, two samples of 96 and 117 villagers from 1 to 85 years of age were administered a radical cure of quinine (Quinimax®; Sanofi, Gentily, France; 25 mg/kg/day divided into 3 equal oral doses administered every 8 hr over a 7-day period by a medical field worker or a physician). They were then monitored weekly for 14 weeks for malaria parasitemia. In addition, each subject was visited daily at home for clinical surveillance and additional thick blood films were made in case of fever. Villagers were allocated at random either to the first or second study to limit the impact of treatment on malaria transmission and parasite diversity in the village. Only those who planned to stay permanently in the village during the whole surveillance period were enrolled in the study. Thick blood films were stained with Giemsa. Those collected during fever episodes were examined immediately and malaria treatment was given according to criteria based mainly on parasite density and age.[10,11] Other blood films were examined at the end of the study. A total of 200 microscopic oil-immersion fields were examined on each slide (about 0.5 μl of blood).

Biologic tests, including hemoglobin electrophoresis, HLA typing, and tests for glucose-6-phosphate dehydrogenase (G6PD) deficiency, were available for most subjects included in the study. The sickle cell trait, the HLA-B53 phenotype, the HLA-DR13 phenotype, and G6PD deficiency were common in the study population. These host genetic factors, which are known to confer protection against severe malaria, were included in the analysis of the results. Type of housing, the location of the house in the village, and the use of bed nets were noted for each person. Malaria transmission was monitored during the study.[12,13] Night-bite collections of mosquitoes landing on human volunteers were carried out during the first week of each month (12 person-nights of capture each month). Anopheline vectors were dissected and examined for sporozoites. The species of *Plasmodium* were determined using species-specific monoclonal antibodies to circumsporozoite protein. The average entomologic inoculation rate (EIR) was estimated to be at 46.1 and 38.4 infective bites per person during the periods January–April and August–November, respectively, and *P. fal-*

TABLE 1

Characteristics of the study population and its distribution according to the week of reappearance of *Plasmodium falciparum* parasitemia

	Reappearance weeks 1–7 (%)	Reappearance weeks 8–14 (%)	Negative weeks 1–14 (%)	Total
Season				
January–April	51 (61)	20 (24)	13 (15)	84
August–November	56 (63)	27 (30)	6 (7)	89
Age (years)				
1–2	13 (87)	1 (6.5)	1 (6.5)	15
3–6	35 (92)	2 (5)	1 (3)	38
7–14	27 (60)	16 (36)	2 (4)	45
≥15	32 (43)	28 (37)	15 (20)	75
Sex				
Male	48 (59)	23 (28)	10 (12)	81
Female	59 (64)	24 (26)	9 (10)	92
Parasite/leukocyte ratio before treatment				
No parasites	21 (43)	18 (37)	10 (20)	49
<0.01	25 (50)	17 (34)	8 (16)	50
0.01 ≤–<0.1	22 (73)	7 (23)	1 (4)	30
0.1 ≤–<1	27 (84)	5 (16)	0 (0)	32
≥1	12 (100)	0 (0)	0 (0)	12
Bed net use				
No	59 (69)	20 (23)	7 (8)	86
Yes	48 (55)	27 (31)	12 (14)	87
Hemoglobin*				
AA	97 (63)	42 (27)	16 (10)	155
AS	8 (50)	5 (31)	3 (19)	16
AC	1 (100)	1 (100)	0 (0)	1
G6PD†				
Normal	90 (61)	39 (27)	18 (12)	147
Deficiency	16 (67)	7 (29)	1 (4)	24
HLA-B53‡				
Absent	75 (61)	31 (25)	17 (14)	123
Present	27 (63)	14 (32)	2 (5)	43
DR13§				
Absent	90 (65)	35 (25)	14 (10)	139
Present	16 (52)	10 (32)	5 (16)	31

* One person not tested.
† G6PD = glucose-6-phosphate dehydrogenase. Two persons not tested.
‡ Seven persons not tested.
§ Three persons not tested.

ciparum was identified in 94% of the infected mosquitoes. For both study periods, similar patterns of variation of the EIR were observed, with maximum values during the 2 first months of each study (January = 19.7–February = 13.1; August = 15.5–September = 13.9) and minimum values during the two last months (March = 9.7–April = 3.6; October = 3.2–November = 5.8).

The cumulative incidence of new patent infections as a function of time was graphically represented using the Kaplan-Meier method. Because of departure from the assumption of proportional hazards, the differences in delay of reappearance of parasitemia between age groups were tested by the Kruskal-Wallis test and the effects of the other variables were tested using an age-stratified Cox model.

The study protocol and objectives were carefully explained to the assembled village population. Informed consent was obtained individually from all participants or their parents. Approval for the study was obtained from the Ministry of Health of Senegal.

TABLE 2

Gender and bed net use distribution of the study population by age groups

Age (years)	Gender (%)		Bednet use (%)		Total
	Male	Female	No	Yes	
1–2	5 (33)	10 (67)	6 (40)	9 (60)	15
3–6	25 (66)	13 (34)	18 (47)	20 (53)	38
7–14	22 (49)	23 (51)	27 (60)	18 (40)	45
≥15	40 (53)	35 (47)	35 (47)	40 (53)	75

RESULTS

A total of 193 of 213 subjects received a full course of treatment, of which 173 were monitored during the entire 14-week follow-up period. The characteristics of these subjects for the variables investigated are shown in Tables 1 and 2. Among them, 124 (72%) were parasite positive before treatment, all except one were negative during the first week after the full course of treatment, and 157 (91%) had new patent infections between week 1 and week 14. *Plasmodium falciparum* was the most prevalent species before treatment and first or only new infections were generally due to this species (*P. falciparum* = 151, *P. ovale* = 3, *P. malariae* = 3). In three cases, the only new infection was due to *P. malariae* (2 cases) or *P. ovale* (one case). Figure 1 shows that incidence rates were similar during the two studies. Their data were combined for further analysis and only *P. falciparum* is considered below. Data from children 1–2 years and 3–6 years of age were also combined since they had similar incidence rates (Table 1).

The median time interval before parasite reappearance was 4 weeks (interquartile interval 25–75% [II] = 3–5 weeks), 6 weeks (II = 5–9 weeks) and 8 weeks (II = 6–12 weeks) among people 1–6 years, 7–14 years, and ≥ 15 years of age, respectively ($P < 0.0001$, by Kruskal-Wallis test). The weekly risks of parasite reappearance in the different age groups were not proportional after the ninth week (Figure 2). During the first 9 weeks, this risk decreased with age ($P < 0.001$) and was 2.5 (95% confidence interval [CI] =

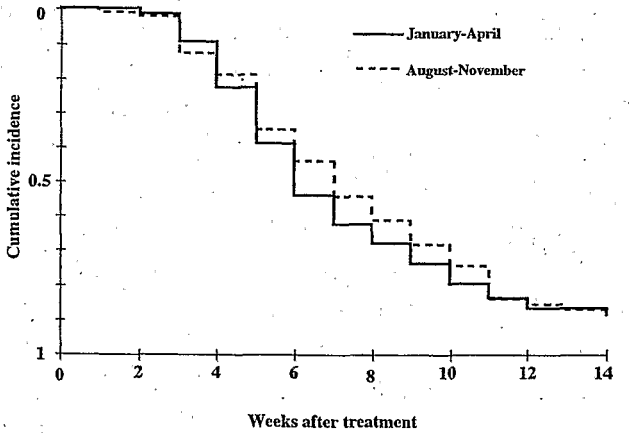

FIGURE 1. *Plasmodium falciparum* cumulative incidence after treatment as a function of time during the two study periods.

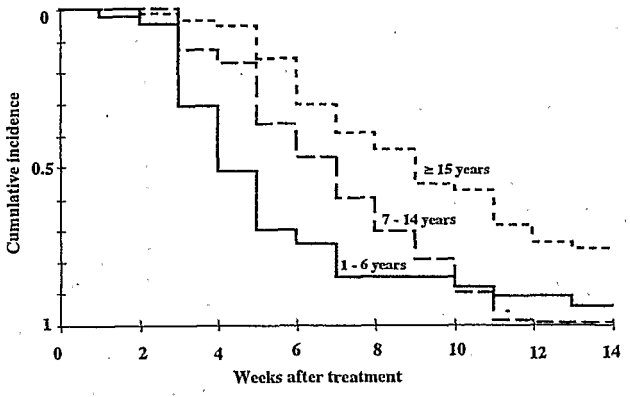

FIGURE 2. *Plasmodium falciparum* cumulative incidence after treatment as a function of time according to age groups.

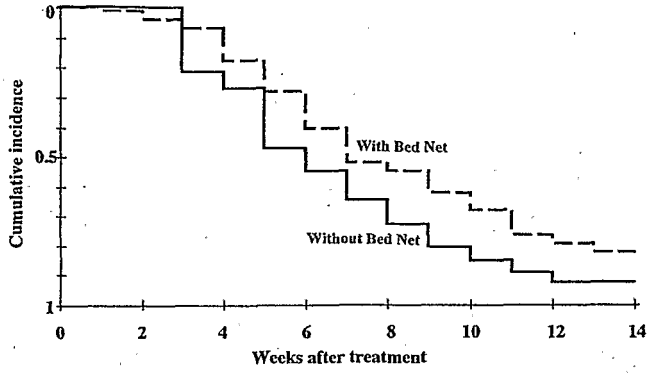

FIGURE 3. *Plasmodium falciparum* cumulative incidence after treatment as a function of time according to the use of bed nets.

1.5–3.9) and 2.8 (95% CI = 1.8–4.6) times lower in those 7–14 years and ≥ 15 years of age, respectively, than in children 1–6 years of age. The delay of reappearance was longer in persons using a bed net than in non-users (Figure 3; $P < 0.01$, by Mann-Whitney test) and in sickle cell trait carriers than in AA individuals (Figure 4; $P = 0.06$, by Mann-Whitney test). The higher the parasitemia before treatment, the earlier reappearance occurred ($P < 0.01$, by Spearman correlation coefficient test). There was no significant association between the duration of this delay and either gender, G6PD activity, HLA-B53 phenotype, HLA-DR13 phenotype, type of housing, or location of the house in the village ($P > 0.25$ for each of these parameters).

Variables included in an age-stratified Cox model for a multivariate analysis were bed net use, sickle cell trait, pretreatment parasitemia, and study period. Independently of age and other factors, the weekly risk of parasite reappearance was 2.2 (95% CI = 1.2–4.5) times lower in sickle cell trait carriers than in AA individuals ($P < 0.02$) and 1.5 (95% CI = 1.1–2.1) times lower in bed net users than in non-users ($P < 0.01$). A 1,000 trophozoites/μl increase in the level of pretreatment parasitemia was associated with a 1.6 (95% CI = 1.2–2.1) risk ratio for the weekly risk of parasite reappearance ($P < 0.001$).

DISCUSSION

In this study, we used a 7-day regimen of quinine to clear malaria parasitemia. Since quinine has the advantage over other antimalarial drugs of being fully effective for the isolates from the study area,[14,15] and of having a very short half-life in the body, the outcome of new infections after the end of treatment would not be influenced. Thus, reappearance of malaria parasites after treatment was likely to correspond to recently inoculated sporozoites, particularly in the case of *P. falciparum* and *P. malariae,* in which most hepatic forms are short-lived. Results suggest that most of the study subjects successfully resisted a large number of sporozoite inocula before presenting a new detectable infection. This may be due to host resistance either at the pre-erythocytic level or at the early blood stage level. In a human, 8–14 days generally transpire between *P. falciparum* sporozoite inoculation by a mosquito and the time of a first detectable parasitemia. The median delay of *P. falciparum* reappearance was 38 days, and this theoretically corresponded to 14 consecutive challenges by infected mosquito bites during the period starting on day 0 after the end of treatment and ending 2 weeks before parasite reappearance. Our data show that age was a major factor affecting this delay: on average, adults resisted significantly more challenges (20) than older children (13) and young children (7). However, it is likely that differences in resistance between age groups were even greater since transmission was measured from collections of mosquitoes landing on adults male volunteers who are more exposed to mosquito bites than young children.[16] Furthermore, bed nets were used by half of the villagers enrolled in the study. Although these bed nets were not insecticide impregnated and were frequently in poor condition, the longer delay in persons using a bed net that in non-users suggests that bed nets significantly reduced exposure to infective mosquito bites. For these reasons, we believe that most young children resisted less than 3 or 4 challenges before successful infection. Differences between age groups probably reflect the high level of anti-parasite immunity that is acquired by individuals continuously exposed to intense perennial malaria transmission.

Reappearance of *P. falciparum* was significantly delayed in individuals with the sickle cell trait compared with AA individuals. Since the study of Allison,[17] numerous studies have documented protection afforded by sickle cell trait against malaria. However, to our knowledge, the present study was the first to investigate the influence of sickle cell

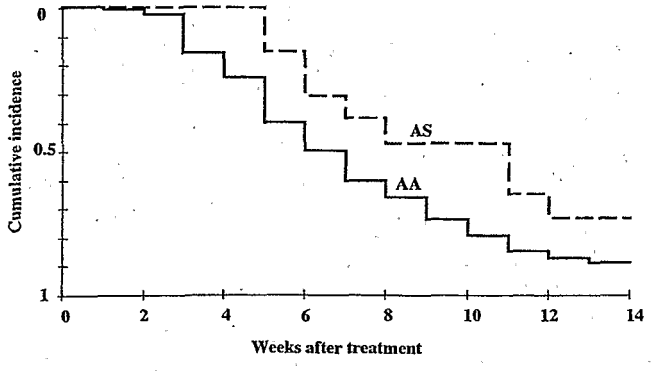

FIGURE 4. *Plasmodium falciparum* cumulative incidence after treatment as a function of time in sickle cell trait carriers and in individuals with the AA hemoglobin genotype.

trait on the delay of reappearance of malaria parasites after radical treatment. A possible explanation of our observations is that individuals with the sickle cell trait are resistant to certain genetic types of *P. falciparum,* an hypothesis that is also consistent with the imbalanced distribution of merozoite surface protein-1 genotypes related to the sickle cell trait that was recently reported in the same population.[18] In contrast, no effect of HLA-B53 and DR13 were observed. Hill and others[19] reported that severe manifestations of malaria were significantly less frequent in people expressing these antigens. They further identified cytotoxic T lymphocytes specific for a highly conserved motif of the liver-stage antigen-1 and proposed that MHC class I-restricted cytotoxic T lymphocytes specific for a malaria peptide able to associate preferentially with B53 could be responsible for an increased defense against malaria in such individuals.[20] Our results do not support the hypothesis that HLA-B53-restricted immune responses against liver-stage antigens would reduce the proportion of new infections emerging from the liver.

Parasite density at enrollment was an important factor affecting the delay of reappearance of *P. falciparum,* and this was independent of age and transmission season. A similar observation was reported from Kenyan children exposed to intense transmission.[6] Most known factors likely to influence the rate and density of parasitemia were controlled in our study. We believe that unknown genetic and/or immunologic factors could be responsible for high susceptibility to malaria, and thus associated with both increased parasite density when infected and rapid reappearance of malaria parasites after treatment.

We conclude that the delay of reappearance of malaria parasites following radical treatment is an useful indicator of anti-parasite immunity, as shown by the major differences between age groups in our study. It may reflect immunity both against pre-erythrocytic stages and early blood stages. The fact that monitoring a small cohort of Dielmo villagers for a few weeks was able to detect differences between subgroups of individuals is of considerable importance for the design of field vaccine trials since it suggests that small, low-cost trials may be very informative on the potential of new vaccine candidates. However, during such trials, the possible confounding effects of host genetic factors and individual differences in exposure to transmission must be controlled.

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REFERENCES

1. Hoffman SL, Oster CN, Plowe CV, Woollett GR, Beier JC, Chulay JD, Wirth RA, Hollingdale MR, Mugambi M, 1987. Naturally acquired antibodies to sporozoites do not prevent malaria: vaccine development implications. *Science 237:* 1639–1642.
2. Dieye A, Rogier C, Trape JF, Sarthou JL, Druilhe P, 1997. HLA class I-associated resistance to severe malaria: a parasitological re-assessment. *Parasitol Today 13:* 48–49.
3. McElroy PD, Beier JC, Oster CN, Beadle C, Sherwood JA, Oloo AJ, Hoffman SL, 1994. Predicting outcome in malaria: correlation between rate of exposure to infected mosquitoes and level of *Plasmodium falciparum* parasitemia. *Am J Trop Med Hyg 51:* 523–532.
4. Beier JC, Oster CN, Onyango FK, Bales JD, Sherwood JA, Perkins PV, Chumo DK, Koech DV, Whitmire RE, Roberts CR, Diggs CL, Hoffman SL, 1994. *Plasmodium falciparum* incidence relative to entomologic inoculation rates at a site proposed for testing malaria vaccines in western Kenya. *Am J Trop Med Hyg 51:* 529–536.
5. Beadle C, McElroy PD, Oster CN, Beier JC, Oloo AJ, Onyango FK, Chumo DK, Bales JD, Sherwood JA, Hoffman SL, 1995. Impact of transmission intensity and age on *Plasmodium falciparum* density and associated fever: implications for malaria vaccine trial design. *J Infect Dis 172:* 1047–1054.
6. McElroy PD, Beier JC, Onyango FK, Oloo AJ, Beadle C, Hoffman SL, 1997. Dose and time-dependent relations between infective *Anopheles* inoculation and outcomes of *Plasmodium falciparum* parasitemia among children in western Kenya. *Am J Epidemiol 145:* 945–956.
7. WHO, 1997. *Guidelines for the Evaluation of Plasmodium falciparum Vaccines in Populations Exposed to Natural Infection.* Geneva: World Health Organization. TDR/MAL/VAC/97.
8. Trape JF, Rogier C, Konaté L, Diagne N, Bouganali H, Canque B, Legros F, Badji A, Ndiaye G, Ndiaye P, Brahimi K, Faye O, Druilhe P, Pereira da Silva L, 1994. The Dielmo project: a longitudinal study of natural malaria infection and the mechanisms of protective immunity in a community living in a holoendemic area of Senegal. *Am J Trop Med Hyg 51:* 123–137.
9. Rogier C, Ly AB, Tall A, Cissé B, Trape JF, 1999. *Plasmodium falciparum* clinical malaria in Dielmo, a holoendemic area in Senegal: no influence of acquired immunity on initial symptomatology and severity of malaria attacks. *Am J Trop Med Hyg 60:* 410–420.
10. Trape JF, Peelman P, Morault-Peelman B, 1985. Criteria for diagnosing clinical malaria among a semi-immune population exposed to intense and perennial transmission. *Trans R Soc Trop Med Hyg 79:* 435–442.
11. Rogier C, Commenges D, Trape JF, 1996. Evidence for an age-dependent pyrogenic threshold of malaria parasitemia in individuals continuously exposed to *Plasmodium falciparum. Am J Trop Med Hyg 54:* 613–619.
12. Konaté L, Diagne N, Brahimi K, Faye O, Legros F, Rogier C, Petrarca V, Trape JF, 1994. Biologie des vecteurs et transmission de *Plasmodium falciparum, P. malariae* et *P. ovale* dans un village de savane d'Afrique de l'Ouest (Dielmo, Sénégal). *Parasite 1:* 325–333.
13. Fontenille D, Lochouarn L, Diagne N, Sokhna CS, Lemasson JJ, Diatta M, Konate L, Faye F, Rogier C, Trape JF, 1996. High variations of malaria transmission and vector species composition in Dielmo, a holoendemic area in Senegal. *Am J Trop Med Hyg 56:* 247–253.
14. Pradines B, Rogier C, Fusai T, Tall A, Trape JF, Doury JC, 1998. *In vitro* activity of artemether against African isolates (Senegal) of *Plasmodium falciparum* in comparison with standard antimalarial drugs. *Am J Trop Med Hyg 58:* 354–357.
15. Rogier C, Brau R, Tall A, Cisse B, Trape JF, 1996. Reducing the oral quinine-quinidine-cinchonine (Quinimax®) treatment of uncomplicated malaria to 3 days does not increase the recurrence of attacks among children living in a highly endemic area of Senegal. *Trans R Soc Trop Med Hyg 90:* 175–178.

16. Carnevale P, Frezil JL, Bosseno MF, Le Pont F, Lancien J, 1978. Etude de l'agressivité d'*Anopheles gambiae* A en fonction de l'âge et du sexe des sujets humains. *Bull World Health Organ 56:* 147–154.
17. Allison AC, 1954. Protection afforded by sickle cell trait against subtertian malarial infection. *Br Med J 1:* 290–294.
18. Ntoumi F, Rogier C, Dieye A, Trape JF, Millet P, Mercereau-Puijalon O, 1997. Imbalanced distribution of *Plasmodium falciparum* MSP-1 genotypes related to sickle-cell trait. *Mol Med 9:* 581–592.
19. Hill AVS, Allsopp CE, Kwiatowski D, Anstey NM, Twumasi P, Rowe PA, Bennett S, Brewster D, McMichael AJ, Greenwood BM, 1991. Common west African HLA antigens are associated with protection from severe malaria. *Nature 352:* 595–600.
20. Hill AVS, Elvin J, Willis AC, Aidoo M, Allsopp CEM, Gotch FM, Gao XM, Takiguchi M, Greenwood BM, Townsend ARM, McMichael AJ, Whittle HC, 1992. Molecular analysis of the association of HLA-B53 and resistance to severe malaria. *Nature 360:* 434–439.

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