

DO AFRICAN IMMIGRANTS LIVING IN FRANCE HAVE LONG-TERM MALARIAL IMMUNITY?

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Abstract. Among populations living in areas endemic for malaria, repeated parasite exposure leads to a gradual increase in protective immunity to the disease. In contrast, this immunity is assumed to disappear after several years of non-exposure. This study was designed to investigate long-term immunity in subjects removed from the risk of exposure. *Plasmodium falciparum* malaria attacks occurring after short trips to sub-Saharan Africa were compared between 99 European patients and 252 African immigrants who had been resident in Europe for at least four years. Relative to the European patients, those originating from Africa had lower mean \pm SD parasite densities ($0.8 \pm 1.5/100$ red blood cells versus $1.4 \pm 2.8/100$ red blood cells; $P = 0.007$), less frequent severe disease (4.4% versus 15.2%; $P = 0.0005$), accelerated parasite clearance and defervescence, and higher levels of antibodies to *P. falciparum*. These results suggest the persistence of acquired immunity to *P. falciparum* malaria after several years of non-exposure in African immigrants.

INTRODUCTION

Malaria kills more than one million people yearly, and more than 40% of the world population is exposed to infection. The annual incidence of clinical malaria attacks is estimated to be 300–500 million; most cases are due to *Plasmodium falciparum* and occur in sub-Saharan Africa.¹ Repeated parasite exposure in endemic regions gradually reinforces protective immunity that reduces the risk of severe malaria.^{2,3} This protection is generally thought to disappear within a few months or years of non-exposure, although this has never been definitely established.^{4–7}

Immigrants account for a large proportion of cases of imported malaria in France. For example, among the 7,000 cases of imported malaria that occurred in France in 1999, 83% were due to *P. falciparum* and 63% involved immigrants.⁸ We formulated the hypothesis that if protection actually disappears within a few months or years of non-exposure, then Europeans (naive individuals) and Africans living in Europe (individuals previously exposed to malaria) will behave similarly during a malaria attack. Therefore, we investigated the possible long-term persistence of immunity despite cessation of exposure by comparing malaria attacks between European and African patients presenting with attacks of *P. falciparum* malaria following short stays in Africa.

PATIENTS AND METHODS

Patients. Patients were recruited prospectively from January 1993 to February 1999 in the Department of Infectious and Tropical Diseases of a teaching hospital (Bichat Claude Bernard Hospital) in Paris, France. They were enrolled in the present study if they 1) were infected with *P. falciparum*; 2) were European or originated from sub-Saharan Africa and had resided in France for at least four years, 3) were infected during a short trip (less than three months) to sub-Saharan Africa, and 4) were at least 15 years old. *Plasmodium falciparum* malaria attacks were defined by the presence of fever and other clinical signs of malaria, and by the detection of asexual stages of *P. falciparum* on thin blood smears.

Plasmodium falciparum density in blood was expressed as the percentage of parasitized red blood cells. Severe and com-

plicated malaria was defined according to 1990 World Health Organization (WHO) criteria and its diagnosis required at least one major criterion or two minor criteria.⁹

Treatment and follow-up. Each patient was seen before and after treatment by one of the study physicians, who collected standardized medical and biologic data. African patients were asked to state the frequency of travel to their home country, and the date of the last trip. The decision to hospitalize a patient was taken by the individual physicians according to clinical and biologic findings. Treatment was administered as recommended by the French Ministry of Health at the time of diagnosis. Uncomplicated malaria was treated with halofantrine (500 mg every six hours for three doses) or with oral quinine (8 mg/kg three times a day for seven days) if halofantrine was contraindicated. Patients with severe or complicated malaria, and those who vomited, were given intravenous quinine. Clinical follow-up included a full physical examination daily, and temperature measurement twice a day. Blood smears were examined every day until parasite clearance, and systematically on day 7. The time required for parasite clearance was calculated from the beginning of specific treatment until the disappearance of asexual forms from thick blood films. The clearance of fever was calculated from the outset of specific treatment until a temperature $\leq 37.2^\circ\text{C}$ (99°F) was maintained for at least 24 hours. *In vitro* chloroquine activity was assessed on blood samples before treatment. Antibody levels to *P. falciparum* were measured using an immunofluorescence method using a thick blood smear of mature asexual stages from an *in vitro* culture of *P. falciparum*.¹⁰ This study was reviewed and approved by the hospital ethical committee, and informed consent was obtained from all patients (or the accompanying person).

Statistical analysis. For univariate analysis, the chi-square test and Fisher's exact test, when necessary, were used to compare the distribution of qualitative variables between patient groups. Continuous variables were compared using a *t*-test. Data were analyzed using Epi-Info software version 6 (Centers for Disease Control and Prevention, Atlanta, GA). *P* values < 0.05 were considered significant. Multivariate analyses (logistic and linear regressions) were performed using BMDP software (BMDP Statistical Software, Inc., Los Angeles, CA) procedures 2R and LR.

RESULTS

Patients. Of the 588 patients seen for malaria attacks during the study period, 351 patients met the inclusion criteria, including 252 African and 99 European patients. A total of 237 patients with a malaria attack were not included because they did not fulfilled one or more of the inclusion criteria: malaria attack not due to *P. falciparum* (n = 63), not originating from Europe or Africa (n = 13), Africans having spent less than four years in France (n = 45), a trip longer than 90 days (n = 91) or not in sub-Saharan Africa (n = 14), and an age less than 15 years (n = 11). Most (90%) of the African patients originated from west or central Africa. The African patients' median length of residence in France was 14 years (range = 4–45 years). The median duration of the stay in the endemic country was 30 days (range = 2–90 days). Two hundred (79.4%) African patients stated the frequency with which they had returned to their native country since they had resided in France: the median frequency was once every five years (range = never in 11.5% to yearly in 24.0%). The mean \pm SD age was similar in the European and African groups (35.3 \pm 11.7 versus 34.7 \pm 9.3 years, respectively; $P = 0.6$), and men were similarly over-represented (66.7% versus 61.5%, respectively; $P = 0.4$).

Characteristics of the stays in endemic areas. Table 1 shows the characteristics of the stays in endemic countries and the prophylactic measures used. Both Africans and Europeans were generally infected in west or central Africa (most frequently Cameroon or Côte d'Ivoire). The length of stay in the endemic country was higher among the Africans than the Europeans (mean \pm SD = 38 \pm 21 days versus 23 \pm 14 days; $P < 0.0001$), and stays in urban areas were also more frequent among the Africans (32.9% versus 13.5%; $P = 0.0005$). Prophylactic measures, whatever the type, were more frequently used by Europeans than by Africans.

TABLE 1

Characteristics of the stays and prophylactic measures taken by 351 patients presenting with *Plasmodium falciparum* malaria attack on their return to France

	Africans (n = 252)	Europeans (n = 99)	<i>P</i>
Area of infection (%)			
West Africa	57.6	64.7	
Central Africa	32.2	24.2	
East Africa	10.3	11	
Characteristics of the stay			
Length of stay (days), mean \pm SD	38 \pm 21	23 \pm 14	< 0.0001
Urban (%)*	32.9†	13.5‡	0.0005
Prophylactic measures (%)			
No chemoprophylaxis	55.8	31.6	< 0.0001
Appropriate chemoprophylaxis§	4.4	31.6	< 0.0001
Repellent	9.0¶	33.0#	< 0.0001
Mosquito net	17.1**	28.9††	0.02
Air conditioner	8.6‡‡	14.4††	0.1

* Stay is defined as exclusively urban (no nights spent in rural areas).

† 219 patients.

‡ 89 patients.

§ Prophylaxis was appropriate if it took into account the characteristics of the country visited (i.e., chloroquine resistance), and respected the recommended dosage and duration (journal plus four weeks after return).

¶ 209 patients.

88 patients.

** 211 patients.

†† 90 patients.

‡‡ 210 patients.

Characteristics of malaria attacks. The characteristics of the malaria attacks and treatment measures are shown in Table 2 for the two groups of patients. The interval from symptom onset to diagnosis was similar, as was the proportion of patients who had treated themselves before presenting to the hospital. However, at diagnosis, severe and complicated malaria was less frequent and the mean parasite density was lower in Africans than in Europeans. Hemoglobin levels were lower in Africans, while white blood cell and platelet levels were higher. Treatments were similar in the two groups. Following treatment, fever and parasite clearance times were shorter in Africans than in Europeans.

Multivariate analyses were performed for the four main outcome variables (severe malaria, parasite density, fever clearance time, and parasite clearance time). The results are presented in Tables 3 and 4. They show that after adjustment on all other covariates, severe malaria remained more frequent in Europeans than in Africans, whereas parasite density was more elevated and temperature clearance time was significantly longer. Conversely, the effect of geographic origin on parasite clearance time disappeared when parasite density was taken into account.

Reciprocal antibody titers. Ten to 12 days after onset of symptoms, antibody levels were measured in a similar proportion of African and European patients (59.5% versus 63.6%, respectively; $P = 0.5$). Plasma samples were collected at a similar time after symptom onset in the two groups ($P = 0.3$). Patients with and without serologic tests were similar with regard to disease severity and parasite density at diagnosis. Antibody levels were higher in African than in European patients (Figure 1), and this difference persisted after adjustment on other covariates ($P = 0.0002$, by linear regression). Indeed, 77.3% of Africans had reciprocal titers ≥ 256 , compared with only 52.4% of Europeans ($P = 0.0003$). Median titers among patients originating from Africa did not differ according to the duration of residence in Europe. When adjusted on other variables, there was no relationship between antibody titers and any of the outcome variables.

DISCUSSION

We compared the features of *P. falciparum* malaria attacks, and their outcome on curative treatment, between patients originating from Africa who had lived in France for more than four years, and European patients who had always lived in non-endemic areas. All patients were infected during a short stay in Africa. The African patients had lower parasite densities, less frequent severe and complicated malaria, and more rapid parasite and fever clearance relative to the European patients. These differences were not related to sex, age, or the interval between clinical onset and diagnosis. Ten to 12 days after onset of symptoms, antibody levels to *P. falciparum* were also higher in African than in European patients. Additionally, when compared with Europeans, Africans presented with a shorter interval between return and onset of malaria attack, a lower hemoglobin level, and a longer difference between fever and parasitemia clearance times. Such differences may also reflect a differential susceptibility/reaction to parasite infection.

The African patients reported less frequent use of prophylactic measures against malaria (chemoprophylaxis, repel-

TABLE 2
Pretreatment characteristics, treatment measures, and treatment outcome*

	Africans (n = 252)	Europeans (n = 99)	p
Pretreatment characteristics			
Time to diagnosis (days)	7 ± 14	7 ± 10	0.9
Inappropriate self-treatment (%)	29.3	24.2	0.3
Interval return/onset (days)	5 ± 9	9 ± 12	0.006
Chloroquine resistance (%)	42.0†	57.1‡	0.2
White blood cells (10 ⁹ /L)	5.5 ± 1.8	4.9 ± 1.5	0.01
Hemoglobin (g/dL)	12.8 ± 1.7	13.6 ± 1.6	0.0003
Platelets (10 ⁹ /L)	119.6 ± 55.7	105.7 ± 59.2	0.04
Temperature (°C)	38.7 ± 1.1	38.8 ± 1.2	0.5
Parasite density (/100 red blood cells)	0.8 ± 1.5	1.4 ± 2.8	0.007
Severe and complicated malaria (%)	4.4	15.2	0.0005
Treatment measures (%)			
Hospitalization	84.1	77.6	0.1
Halofantrine	57.4	56.1	0.8
Quinine	32.3	31.6	0.9
Others	10.4	12.2	0.6
Disease outcome			
Fever clearance (hours)	40.1 ± 24.6§	56.1 ± 31.2¶	< 0.0001
Parasitemia clearance (hours)	54.6 ± 24.0#	62.5 ± 30.5**	0.03

* Values are the mean ± SD where indicated.

† 81 patients.

‡ 21 patients.

§ 220 patients.

¶ 77 patients.

189 patients.

** 67 patients.

lents, bed nets, and air conditioning) than European patients during their stay in Africa. As suggested by these differences in the characteristics of the stay and in the socioeconomic status, the Europeans can be expected to have better housing and living conditions in which there are less exposed to mosquito bites compared with the Africans, and that they might therefore have received a larger inoculum of *P. falciparum* sporozoites. Although the latter is thought to be related to more severe disease, the African patients were less likely than the Europeans to have severe and complicated malaria.^{11–13} Conversely, the fact that Africans spent more time exclusively in an urban environment (where sporozoite exposure is often lower than in rural areas) than the Europeans might produce the opposite result.

In our study, the rate of severe and complicated malaria is higher than that usually reported in imported malaria.^{14,15} Such a difference can be explained by the high prevalence of *P. falciparum* malaria imported in France and because we used the 1990 WHO classification including major and minor criteria. A high proportion of patients had two minor criteria such as obnubilation, icterus, hyperbilirubinemia, or hyperparasitemia. This could lead to an over-estimation of severe forms. Nevertheless in two studies comparing data between Africans and Europeans, a lower prevalence of complicated

malaria is found in Africans (3.7% versus 6.3% and 1.3% versus 9.2%, respectively) as in our report.^{14,15}

Several other factors may explain this difference in malaria disease expression between African immigrants and Europeans, including differences in their genetic background, or differences in the *P. falciparum* strains infecting the two groups. Various genetic factors affecting red blood cells, such as the sickle-cell trait, α - and β -thalassemia, and glucose-6-phosphate dehydrogenase deficiency, are more frequent in African populations and have been linked to decreased susceptibility to malaria.^{16–20} Unfortunately, the hemoglobin status of our patients was not characterized. In our study population, the sickle cell trait is undoubtedly the most frequent hemoglobinopathy, since it occurs in 15–20% of the population from sub-Saharan Africa. However, even if the trait was fully effective in preventing severe disease, this would achieve a maximum rate of severe and complicated malaria of 5.4% in Africans who do not have the sickle cell trait. Therefore, this cannot account for the difference of this rate when compared with Europeans (15.2%). Alternatively, it has been suggested that selected parasite strains may be more virulent than others.^{13,21} However, since both groups of patients were infected in various parts of Africa over a six-year period, it is unlikely that virulence differed between the two groups. The possible relationship between infection with human immunodeficiency virus (HIV) and a higher severity of malaria cannot be excluded.²² This information was not available in our study, but there is no reason to believe that our findings are due to a higher HIV prevalence in the European patients than in the African patients.

This apparent existence of residual immune memory after several years in a non-endemic area is surprising because malaria immunity has been reported to wane rapidly after the end of exposure to the parasite.^{4–7} However, during the acute phase of the malaria attacks, antibody levels to *P. falciparum* were higher in the African patients than in the European

TABLE 3

Results of logistic regression of severity of malaria on the geographic origin (Europeans versus Africans) and other covariates*

Variable	Odds ratio	95% confidence interval	p
Geographic origin	4.3	1.6–11.9	0.003
Adequate chemoprophylaxis†	0.3	0.03–2.5	0.05‡
Inadequate chemoprophylaxis†	2.4	0.83–6.8	

* Only covariates for which $P < 0.10$ are shown.

† Absence of chemoprophylaxis constitutes the reference group.

‡ Test of the overall effect of chemoprophylaxis (three classes) on severity of malaria.

TABLE 4

Results of linear regression of parasite density, parasite clearance time, and fever clearance time on the geographic origin (European versus Africans), and other covariates*

	Variable	Regression coefficient	95% confidence interval	P
Parasite density	Geographic origin	0.6	0.2–1.0	0.08
Parasite clearance	Parasite density	4.7	3.1–6.3	< 0.0001
Fever clearance	Geographic origin	14.6	7.9–21.3	< 0.0001
	Temperature	10.7	8.0–13.4	< 0.0001

* Only covariates for which $P < 0.10$ are shown.

patients. Given that the antibody level were measured a mean of 10–12 days after symptom onset in both groups, a malaria attack could have boosted immunity in Africans who were exposed prior to residence in France compared with Europeans without previous exposure. Although such antibodies do not by themselves confer protection against malaria, but rather simply indicate previous contact with malarial antigens, this higher antibody level in Africans might be an argument for the existence of residual immune memory. Interestingly, antibody levels during the acute phase were unrelated to the length of residency in Europe.

Thus, when taken together, our data strongly suggest the persistence of a specific immune response in African patients removed from the risk of exposure to *P. falciparum*. Literature on this topic is poor. As previously states, no convincing demonstration is available to support the classic assertion regarding disappearance of immunity within a few months or years of non-exposure. Conversely, the results of a few studies are consistent with our findings. An *in vitro* study showed that humoral and cellular responses to defined *P. falciparum* antigens persisted in migrants from west Africa who spent up to 13 years in France without returning to their native country.²³ Two studies conducted in Nigeria and Sudan showed that long-term drug prophylaxis in children did not diminish protective immunity after termination of drug distribution.^{24,25} Moreover, such long-term persistence of anti-malarial immunity is consistent with observations in the highlands of Mada-

gascar (which are a non-endemic area for malaria) during the 1987 malaria outbreak. Individuals more than 40 years old who had spent their childhood in a malaria hyperendemic area before implementation of a control program were protected more against clinical *P. falciparum* malaria than were younger subjects, despite being submitted to a similar risk of infective mosquito bites; they also had stronger humoral and cellular immune responses to *P. falciparum* antigens.²⁶ These differences were attributed to a difference in past exposure to malaria parasites.

Most of the African patients in our study had previously visited their country of origin since migrating to Europe, and these visits, despite their low frequency (once every five years on average) may have contributed to maintenance of long-term immune memory. Nevertheless, we observed no difference between patients who had rarely returned to their native countries and those who had made more frequent visits, although this may have been due to inadequate statistical power.

In conclusion, imported malaria in African adult migrants is less severe (lower parasite density and lower frequency of severe and complicated disease) and more readily cured (shorter parasite and fever clearance times) than it is in Europeans. This difference may be related to long-term persistence of immune memory. However, travel physicians must continue to recommend optimal anti-malaria prophylaxis for all patients visiting endemic areas.

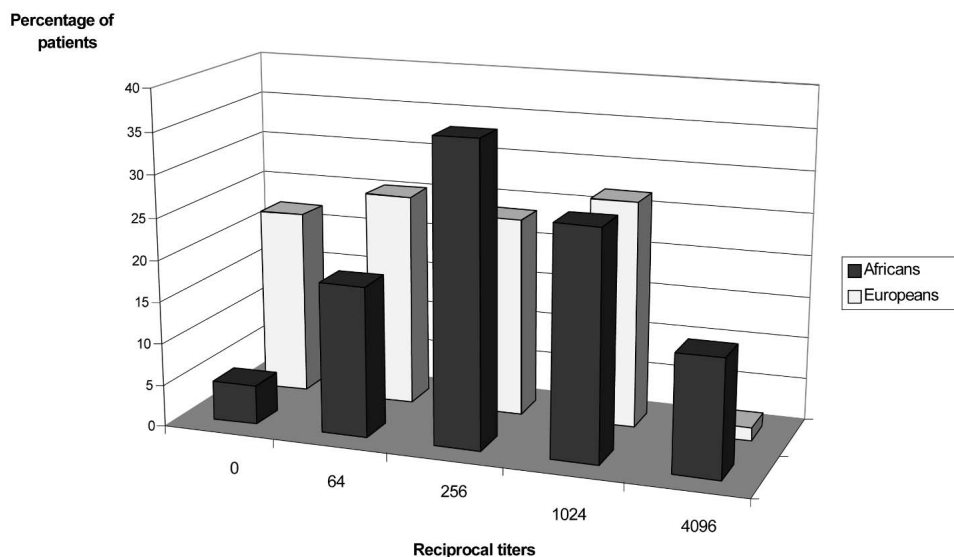


FIGURE 1. *Plasmodium falciparum*-specific antibody titers in Africans (n = 150) and Europeans (n = 63) presenting with a *P. falciparum* malaria attack on their return to France.

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