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EFFECT OF THE SICKLE CELL TRAIT STATUS OF GAMETOCYTE CARRIERS OF *PLASMODIUM FALCIPARUM* ON INFECTIVITY TO ANOPHELINES

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Abstract. Insect-reared *Anopheles gambiae* were experimentally fed with the blood of naturally infected human volunteers carrying gametocytes of *Plasmodium falciparum.* Infection of at least one mosquito was successful in 86 experiments. For these gametocyte carriers, the hemoglobin types studied were AA (normal, n = 77), AS (heterozygous sickle cell, n = 8), and SS (homozygous sickle cell, n = 1). The mean of the percentages of infected mosquitoes by gametocyte carriers of AS hemoglobin was almost double that of carriers of AA: 30.4% versus 17.5%. The genetic protection in humans conferred by the β^S gene in its heterozygous form seems to be associated with an increasing effect on *P. falciparum* transmission from humans to mosquitoes. The epidemiologic and evolutionary aspects of this finding are discussed.

In humans, it is now accepted that sickle cell hemoglobin (HbS) provides selective protection to heterozygote carriers from dying of *Plasmodium falciparum* malaria.[1] Furthermore, transgenic mice expressing HbS are protected from rodent malaria[2] and rodent cerebral malaria.[3] Recent case-control studies in The Gambia[4] and Kenya[5] have indicated that heterozygosity for HbS provides more than 90% protection from both cerebral malaria and severe malarial anemia in children. The effect of hemoglobin HbS on parasite density is less evident.

On the other hand, the possible effect of sickle cell hemoglobin on malaria transmission is unknown. Sexual development of *Plasmodium* begins in the vertebrate host with the invasion of erythrocytes by merozoites, followed by the differentiation of trophozoites into gametocytes, which is the only stage competent to pursue its development in the mosquito. When ingested in a blood meal by the anopheline vector, the development of *Plasmodium* first takes place in the lumen of the mosquito midgut until the ookinete stage, which penetrates through the epithelium of the midgut and transforms into a young oocyst.

The goal of our study was to establish the development of the malarial parasite from naturally infected hemoglobin type AA, AS, and SS individuals when ingested by an anopheline. We postulated that development might be affected by the presence of HbS and consequently have epidemiologic implications in regions where the prevalence of this variant is substantial.

This study was part of broader epidemiologic investigations on malaria transmission, specifically on parasite-vector relationships, in central Africa. Previously, our group has defined the factors influencing the success of the development of the parasite in mosquitoes.,[6,7] as well as their epidemiologic implications.[8]

MATERIALS AND METHODS

Mosquitoes. A strain of *Anopheles gambiae* sensu stricto, caught in 1988 in Yaounde, Cameroon was maintained under laboratory conditions and adapted to feeding on a membrane. Details of rearing have been described previously.[6]

Gametocyte carriers. Thick smears were prepared from blood of patients with malaria-like complaints at the dispensary of Messa, a central quarter in Yaounde. The slides were stained with Giemsa and examined immediately. Patients were informed of the results and gametocyte carriers were asked to cooperate in the study. Consent for children's samples was obtained from their parents.

Blood collection. Intravenous blood was collected into both dry (without heparin) and heparinized vacutainer tubes. The blood from the dry tube was used to prepare Giemsa-stained thick smears. Gametocyte density was based on a count of the number of gametocytes per 1,000 leukocytes, assuming an average number of 8,000 leukocytes/μl. The heparinized blood was used for experimental infection and for hemoglobin determination.

Experimental infections. A membrane feeder with a feeding surface area of 1,134 mm^2 as described by Ponnudurai and others[9] was quickly filled with 2 ml of blood and a variable number of mosquitoes were allowed to take blood through a Parafilm® (American Can Company, Greenwich, CT) membrane for 15 min. Fed mosquitoes were placed into another cage with permanent access to a 10% sucrose solution. After seven days, surviving mosquitoes were dissected. Midguts were stained with a 2% mercurochrome solution and examined for the presence and number of oocysts by light microscopy.

Hemoglobin characterization. The hemoglobin phenotype was determined by cellulose acetate electrophoresis at pH 8.6 (kit; Helena Laboratories, Saint-Leu la Foret, France). The percentage of each type of hemoglobin present was determined by densitometry (Cliniscan; Helena Laboratories, Beaumont, TX) after clearing the cellulose acetate strips. Abnormal hemoglobins were further tested by agar electrophoresis at pH 6.4 and by a solubility test, to excluded other variants (HbD, Lepore, etc.) that migrate as HbS variants on cellulose acetate.

TABLE 1

Results of experimental infections in mosquitoes with blood of 139 volunteers naturally infected with gametocytes of *Plasmodium falciparum**

	Hemoglobin type			
	AA	AS	SS	Total
Number of experimental infections	123	15	1	139
Mean age of carriers	19.1	22.6	23	19.5
Standard deviation	9.5	14.5	—	10.1
Prevalence of trophozoites	69.1	66.7	0	68.3
Mean density of trophozoites	5,689	1,645	0	5,212
Standard deviation	9,265	3,680	—	8,891
Mean density of gametocytes	170.52	107.2	48	162.8
Standard deviation	15.0	93.1	—	205.1
Total number of dissected mosquitoes	4,614	493	42	5,149
Total number of infected mosquitoes	446	68	2	516
% of total infected mosquitoes	97.0	13.8	4.8	10.0
Mean number of dissected mosquitoes	37.5	32.9	42.0	37.0
Mean % of mosquitoes infected	11.0	16.2	4.8	11.5
Standard deviation	16.4	4.8	—	16.8

* — = cannot be calculated (number of observations = 1).

TABLE 2

Results of 86 experiments that infected at least one mosquito*

	Hemoglobin type			
	AA	AS	SS	Total
Number of positive experimental infections	77	8	1	86
Mean density of gametocytes	226.1	155.0	48.0	217.4
Standard deviation	243.1	97.6	—	233.3
Total number of dissected mosquitoes	2,840	220	42	3,012
Total number of infected mosquitoes	446	68	2	516
% of total infected mosquitoes	15.7	30.9	4.8	17.1
Mean % of infected mosquitoes	17.6	30.4	4.8	18.6
Standard deviation	17.8	16.4	—	18.0
Mean number of oocysts per infected midgut	2.57	2.69	1.00	2.56
Standard deviation	2.59	1.46	—	2.49

* — = cannot be calculated (number of observations = 1).

Criteria of enrollment. *Plasmodium falciparum* gametocyte carriers, without other plasmodial species and at least four years of age, were included in the study. Results concerned only experiments in which at least 20 mosquitoes could be examined on day 7 after the experimental infection.

Statistical analysis. Since the Hb SS group consisted of a single individual, she was not included in the analysis. Comparison of the parasite densities among the AA and the AS individuals was done using the Student's *t*-test. Pearson's chi-square test and Fisher's exact probability test were used to compare the proportions of the infected mosquitoes, and the Mann-Whitney U test was used for comparing the mean number of oocysts per infected midgut.

RESULTS

Of 171 experimental infections performed between October 1990 and January 1993, 139 satisfied the enrollment criteria. Forty-nine female and 90 male carriers were used in this study. The distribution of hemoglobin types was 88.5% AA, 10.8% AS, and 0.7% SS (Table 1). The prevalence of trophozoites in AA and AS individuals was not significantly different ($P = 0.53$, by Fisher's exact test); neither was the mean density of asexual forms ($t = 1.669$, degrees of freedom [df] = 1, $P = 0.10$). Gametocytemia was also not significantly different ($t = 1.127$, df = 1, $P = 0.26$).

Sixty-three percent of the carriers of gametocytes with AA hemoglobin infected at least one mosquito versus 53% with AS hemoglobin; this difference was not significant ($\chi^2 = 0.49$, $P = 0.49$). Of the 86 positive infections, the mean of the percentages of infected mosquitoes by AS gametocyte carriers was higher than that of AA individuals: 30.42% versus 17.55% (Table 2) and the difference was significant ($\chi^2 = 34.9$, $P < 10^{-7}$). Stratification of the gametocyte densities did not modify notably the significant differences in mosquito infectivity between AA and AS individuals. The mean number of oocysts per infected midgut was comparable in both the hemoglobin AA and AS groups ($P > 0.9$, by the Mann-Whitney U test). Mosquito infectivity was unrelated to the age and sex of the gametocyte carriers.

Mosquito survival rates between days 0 and 7 in the AA and AS groups were 63% and 57% respectively ($\epsilon = 3.06$, $P < 0.01$) (observation done on 67 AA and 10 AS experimental infections with a total of 4,569 and 609 mosquitoes, respectively).

DISCUSSION

Our results obtained with naturally infected gametocyte carriers in an endemic malarial zone of central Africa clearly demonstrate that *P. falciparum* gametocytes that develop in the red blood cells containing hemoglobin type HbS (AS or SS) are functionally normal and perfectly capable of infecting an anopheline vector.

Although gametocyte density (the most important factor for the infectivity of gametocyte carriers)[6] is almost double in the blood of AA individuals compared with that in AS individuals, the frequency of mosquito-infecting gametocyte carriers is statistically the same in both groups. However, among the infective gametocyte carriers, those carrying AS red blood cells infect almost two times the number of mosquitoes as donors with normal red blood cells. This means that the infective potential of one gametocyte would be four times higher when carried by an individual with AS hemoglobin. This apparent facilitation of the passage of parasites from AS carriers to the mosquito does not result in an increase in the number of oocysts in the midgut of the infected vector. The apparent contradiction between these two results cannot be explained with the available data; however, possible leukocytosis greater than or less than 8,000/μl in malaria patients could make our gametocyte counts an over or underestimation. Despite the statistical significance in mosquito survival rates in a comparison of AA versus AS individuals, the difference was approximately 10% (AA > AS)

and was too small to totally explain the larger difference observed between mosquitoes infected by AA versus AS gametocytes.

Of interest was the observation that some AS or SS red blood cells underwent sickling in the anopheline midgut (Nagel RL and others, unpublished data). Sickling involves most of the red blood cells and modifies the intrastomacal contents when malarial parasites go through major transformations at the zygote-ookinete stages. It is hypothesized that interactions between malarial parasite development and midgut contents might explain the high infectivity of gametocytes in sickled red blood cells.

The genetic protection conferred by the β^S gene in its heterozygous form, reducing the circulating asexual parasitemia, does not have a limiting effect on the transmission of the parasite from humans to mosquitoes. On the contrary, gametocytes from the carriers with AS hemoglobin infected a higher percentage of mosquitoes. Since transmission depends on the number of infected mosquitoes rather than on their parasite loads, this finding suggests an adaptative evolution of the malarial parasite based on a selective advantage. This could have a significant epidemiologic effect by increasing the global level of malaria transmission in countries (as in southern Cameroon) where the prevalence of AS hemoglobin in the population is approximately 20%.[10] Gametocyte carriers with SS hemoglobin are not likely to have an epidemiologic effect due to their very low prevalence.

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