Am. J. Trop. Med. Hyg., 54(1), 1996, pp. 18–26

RAPID TURNOVER OF *PLASMODIUM FALCIPARUM* POPULATIONS IN ASYMPTOMATIC INDIVIDUALS LIVING IN A HIGH TRANSMISSION AREA

PIERRE DAUBERSIES, SELMA SALLENAVE-SALES, SEBASTIEN MAGNE, JEAN-FRANCOIS TRAPE, HUGUES CONTAMIN, THIERRY FANDEUR, CHRISTOPHE ROGIER, ODILE MERCEREAU-PUIJALON, AND PIERRE DRUILHE

Laboratory of Biomedical Parasitology and Unit of Experimental Parasitology, Pasteur Institute, Paris, France; Institut Francais de Recherche Scientifique pour le Developpement en Cooperation (ORSTOM) and Pasteur Institute, Dakar, Senegal

Abstract. A polymerase chain reaction (PCR) typing technique, based on the amplification of polymorphic regions from the merozoite surface protein 1 (MSP-1) and MSP-2 *Plasmodium falciparum* genes, was used to characterize parasites collected in a longitudinal study of asymptomatic carriers of malaria parasites living in two distinct epidemiologic situations. Blood samples were collected from children and adults living in the village of Dielmo, Senegal, when malaria transmission was 3–6 infective bites/week/individual. For each individual, every sample collected at two-week intervals over a period of three months showed a specific PCR pattern. Changes involved both appearance and disappearance of specific alleles. Analysis of blood samples collected at a few-days interval showed that modifications of the PCR patterns occurred rapidly. Most alleles were detected over a period of 2–3 weeks, but some alleles could be detected only for a few days. The frequent modifications of the PCR patterns indicate significant changes in allelic balance over time, and importantly, this was observed both in children and adults. These results strongly contrast with the stability of the parasite types harbored by asymptomatic individuals living in Pikine, Senegal during a period in which malaria transmission was interrupted, and therefore suggest that the rapid turnover observed in Dielmo may reflect the introduction of new parasite populations by mosquitoes.

Protective immunity against *Plasmodium falciparum* malaria is acquired slowly after a large number of infections and its maintenance requires a sustained exposure to infected mosquitoes. Adults living in holoendemic and hyperendemic malaria areas possess a status of premunition, resulting in a high prevalence of individuals carrying low-grade parasitemia without presenting clinical symptoms.[1] In these regions, asymptomatic carriage can be observed in more than 90% of individuals, as demonstrated by highly sensitive detection techniques such as the polymerase chain reaction (PCR).[2] This peculiar immune status is generally described as the successive and/or concomitant involvement of two distinct immune phenomena: 1) a clinical or anti-disease immunity that enables the carriage of important parasite loads in the absence of symptoms and that develops rapidly during childhood, and 2) an anti-parasite immunity, responsible for a marked reduction of the parasite densities after the age of 15–20.[3] It is still unclear how these two mechanisms interact or complement one another and result in asymptomatic carriage of the parasite.

Plasmodium falciparum isolates are known to be composed of complex mixtures of parasite populations, as demonstrated by isoenzyme patterns, two-dimensional gels, monoclonal antibody studies, or PCR analysis of polymorphic markers.[4–6] However, since most samples characterized so far have been collected from symptomatic patients, very little is known about the complexity and behavior of parasite populations harbored by constantly exposed asymptomatic individuals from holoendemic regions. In 1990, an in-depth, follow-up study was initiated on the entire population of the village of Dielmo in Senegal where transmission is high and perennial, thus providing not only precise information on entomologic parameters in this village, but also a documented survey of the epidemiologic, clinical, parasitologic, and immunologic history of each inhabitant.[7] A cross-sectional survey of asymptomatic residents from this village demonstrated that the complexity of the infections decreases in an age-dependent manner in this village, in parallel with the appearance of an immune protection and a reduction of parasite density. Furthermore, this showed that acquired anti-parasite immunity not only reduces parasite density, but also limits the number of parasite genotypes infecting an individual at a given time.[8] However, little is known about how these infections evolve over long periods of time.

We performed a longitudinal analysis of the parasite populations infecting asymptomatic individuals living in Dielmo. We also studied longitudinal samples from chronic carriers living in a low-endemicity urban area during a period of nontransmission. Parasites were characterized using a PCR technique based on amplification of polymorphic merozoite surface protein 1 (MSP-1) block 2 and MSP-2 central domain. The sensitivity and the potential limitations in the analysis of samples containing several alleles have been detailed in a previous report.[9] The data reported here provide new information on the dynamics of host-parasite relationships, on anti-parasite immunity, and on parasite evasion mechanisms.

MATERIALS AND METHODS

Study site, choice of blood donors, and collection of isolates. All blood samples were collected with the informed consent of the donors or their parents. The highly endemic site chosen was the village of Dielmo, Senegal, 280 km southeast of Dakar, where malaria transmission is perennial due to the presence of a stream, but is maximal during the rainy season that occurs between July and October.[7] The first follow-up study was conducted between June and September 1990 (individuals with identification numbers 07/06, 10/05, 11/13, 13/03, and 29/06) and the second one between August

and September 1992 (individuals with identification numbers 06/01, 04/01, and 18/11). During both these periods, the entomologic inoculation rate was 3–6 infective bites/individual/week.[7] Studied subjects were selected on the criteria of a positive thick blood smear at enrollment and the absence of gametocytes during the follow-up study. None of these individuals presented any clinically patent malaria episode in the course of, and for at least one month prior to, the survey.

The third follow-up study was performed in the district of Pikine, Senegal, a Dakar suburb. Samples were collected between January and February 1993, i.e., during a nontransmission period.[10, 11] Blood donors (individuals with identification numbers 407, 512, and 385) were randomly chosen among patients with malaria coming in for consultation in December 1992 who were treated with chloroquine and who showed subsequent asymptomatic chronic carriage of parasites despite treatment.

All samples were collected and stored as previously described.[9] Parasite density was determined by microscopic examination of Giemsa-stained thick blood smears.[12]

Extraction of DNA and PCR conditions. The DNA from approximately 10^9 red blood cells was phenol-extracted as previously described and resuspended in 70 μl of water.[9] Five microliters were amplified according to the manufacturer's recommendations (Amersham, Buckinghamshire, United Kingdom) in an Hybaid automated heat block (Cera-Labo, Ecquevilly, France) for 35 cycles (94°C for 2 sec, 55°C for 1 min, 72°C for 2 min). The PCR analysis used here was based on the amplification of highly polymorphic regions from two markers: block 2 of MSP-1 and the repeated region of MSP-2.[13, 14] The primers used were 1) for MSP-1: MSP-1.A (5′-AAG CTT TAG AAG ATG CAG TAT TGA C-3′) and MSP-1.B (5′-ATT CAT TAA TTT CTT CAT ATC CAT C-3′); and 2) for MSP-2: MSP-2.2 (5′-AAC GAA TTC ATA AAC AAT GCT TAT AAT ATG AGT-3′) and MSP-2.3 (5′-GAT GAA TTC TAG AAC CAT GCA TAT GTC CAT GTT-3′). Detailed procedures are described by Contamin and others.[9]

PCR analysis and hybridization conditions. Ten microliters of PCR mixture were loaded on a 3% Nusieve GTG agarose gel (FMC Bioproducts, Rockland, ME) containing ethidium bromide. The PCR products were blotted on nylon membranes (Hybond-N, Amersham) and hybridized with family-specific probes, namely K1, MAD20, or RO33 for MSP-1, and IND/3D7 or FC27 for MSP-2.[13–15] Generally, relevant family-specific probes were combined before labeling and hybridization, except for the samples collected in 1992 (Dielmo) for which MSP-1 and MSP-2 amplification products were sequentially hybridized with each relevant family-specific probe. Sequences of the probes have been previously verified[9, 16] and are available in most common databanks. Membranes were washed twice in 0.5× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate) and twice in 0.1× SSC at 65°C.

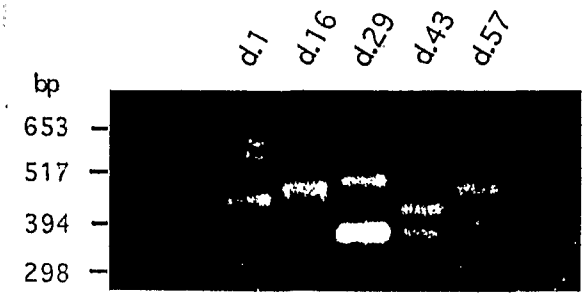
RESULTS

Longitudinal analysis of asymptomatic Dielmo villagers during a period of intense transmission. *Two-week samplings.* We first analyzed the complexity of parasite populations harbored by constantly exposed asymptomatic carriers and the duration of carriage of specific parasite genotypes. Figures 1 and 2 show the MSP-1/MSP-2 PCR products amplified from blood samples collected every 2–4 weeks during the 1990 rainy season from individuals between three and 26 years of age (see figure legends). Both polymorphic markers indicate that these asymptomatic infections were complex, since in some cases up to 5–6 bands could be visualized in one sample with a single pair of primers. In all cases, the PCR patterns obtained with the samples collected from a single individual were all distinct from one another, indicating substantial changes in the composition of the parasite populations in the peripheral circulation. It is important to stress that the modifications of these PCR patterns involved both the appearance and disappearance of certain amplified products, which were not related to modifications in the parasite densities. A typical example is shown in Figure 1, in which samples from a 3.5-year-old child have been analyzed. The 440-basepair (bp) band amplified from the sample collected on day 1 was no longer detected in the next sample collected on day 16. The amplification of two new bands (470 bp and 525 bp) on day 16, while the parasite density on that day was lower than on day 1, indicates that the dominant parasite genotypes on day 16 were different from those prevailing on day 1. The detection of these two new bands reflects either recent inoculation(s) or an increase in the parasites that had previously been carried at a lower density. The two bands amplified from the sample collected on day 16 could no longer be detected in the samples collected on day 29 and day 43, which reflects at least a significant decrease in the density of the corresponding parasites. Likewise, the amplification of two novel alleles (360 bp and 480 bp) from the sample collected on day 29, concomitant with a four-fold increase in parasitemia, can be attributed to either a novel infection or a significant increase in the parasites previously carried at a lower density. The 360-bp band was still detected, with a lesser intensity, on day 43 although it could not be detected on day 57. Additionally, two new genotypes (420 bp and 515 bp) were detected on day 43. Since they were absent from the products generated from the samples collected previously, it is likely that they resulted from a recent inoculation. Similarly, on day 57, three novel parasite populations were visualized, while the three former ones were no longer amplified. The comparison between the profiles obtained on day 43 and day 57 is particularly informative, since the parasite densities were similar and, therefore, the distinct patterns obtained reflect totally different parasite populations. This clearly suggests a rapid turnover of the parasites detectable in the peripheral circulation.

Similarly, rapidly changing patterns were observed in each of the four individuals analyzed in Figure 2. Indeed, both MSP-1 and MSP-2 profiles differed from one sample to the next. Note that parasite densities in the samples from individuals 07/06, 10/05 and 11/13 were in the same range. This further suggests that the modifications observed reflect intrinsic differences in the parasite populations present at the various time points in the same individual.

Sampling at 2–4-day intervals. Our results led us to investigate in greater detail the turnover rate of the parasite populations present in asymptomatic individuals. A second longitudinal study with samples taken at shorter time inter-

A



B

day of sampling	1	16	29	43	57
paras./100 leukocytes	108	29	126	56	65

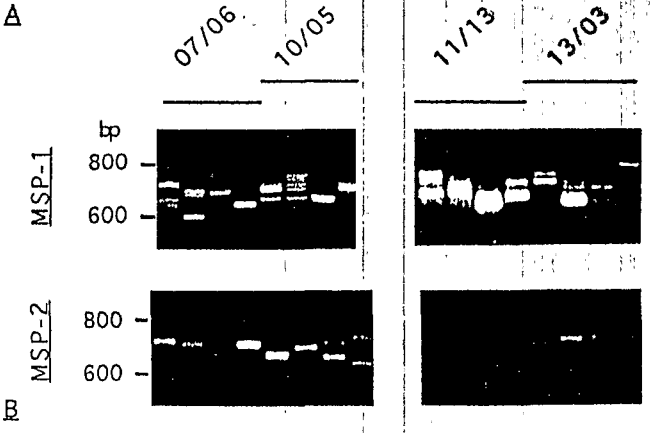
FIGURE 1. **A**, merozoite surface protein 1 (MSP-1) (block 2) amplifications and **B**, corresponding parasite densities of consecutive samples from an asymptomatic 3.5-year-old child from Dielmo, Senegal (individual code 29/06) collected five times over a period of 57 days. bp = basepairs; paras. = parasites.

vals was thus performed in Dielmo between August and September 1992, a similarly high transmission season (Trape J-F, unpublished data). One teenager (18/11) and two adults (06/01 and 04/01) were enrolled. For these three individuals, a series of blood samples was obtained every 2–4 days. To obtain more precise information on allelic carriage, each gel was blotted and sequentially hybridized with the relevant family-specific probes (Figures 3–5). As expected, these hybridizations led to the visualization of bands not detected by staining with ethidium bromide.

An example of the least complicated situation is shown in Figure 3, where five distinct subpopulations could be visualized using MSP-1 typing over a period of 24 days. Two different 480-bp alleles, one MAD20-type and one RO33-type, were detected during the survey and their intensity fluctuated, presenting a peak from day 14 to day 19, concomitant with a significant increase in parasitemia. From day 1 to day 6, a 540-bp K1 allele was also amplified and was no longer detectable thereafter. From day 3 on, a novel, 430-bp K1 allele was detected and was still amplified at the end of the survey. The intensity of the amplification fragments peaked on days 12–19. On day 9, although the parasitemia was equivalent to that of day 6, a new 430-bp RO33 allele was observed, although this was no longer amplified from the following samples. It is noteworthy that the peak of parasitemia between day 12 and day 19 was accompanied by a simultaneous increase in the intensity of the three alleles detected during this week.

The MSP-2 amplifications obtained from samples collected from an asymptomatic teenager shown in Figure 4 revealed some of the features described for the previous case. Indeed, detection of a 640-bp FC27 allele fluctuated from day 12 to day 39. Another FC27 allele (730 bp) was also detected with varying intensity from day 12 to day 29 and was no longer detected thereafter, while a new IND/3D7 allele of the same size was amplified. Another IND/3D7 al-

A



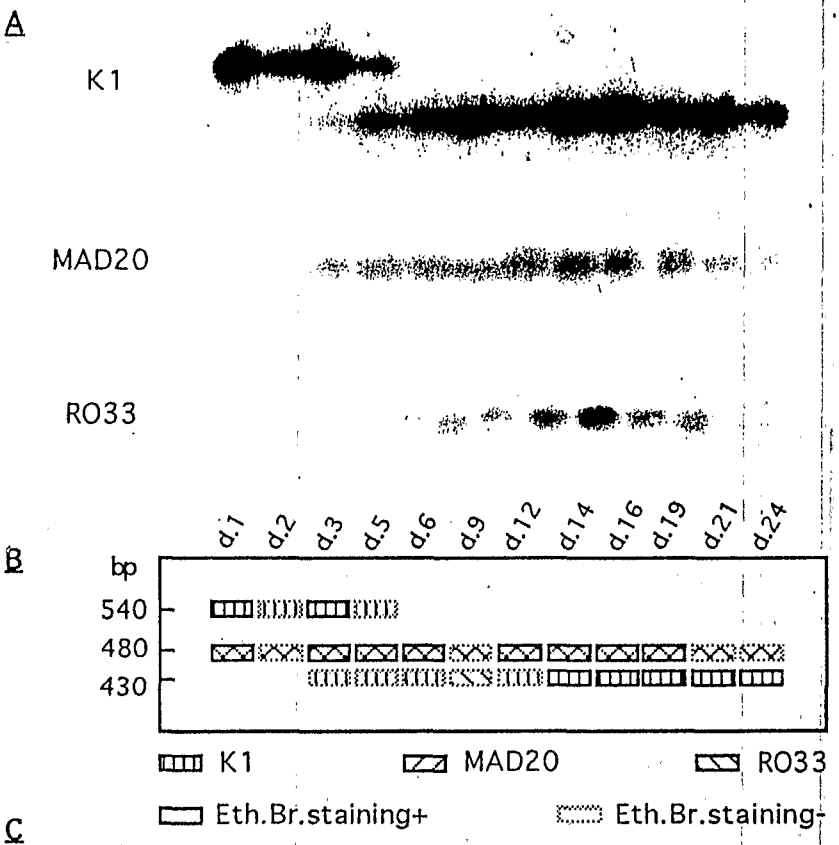
B

individ.	07/06				10/05			
day of sampling	1	30	57	71	1	30	53	81
paras./100 leukocytes	27	12	7	36	5	16	9	8
individ.	11/13				13/03			
day of sampling	1	30	57	85	1	26	36	81
paras./100 leukocytes	15	n.a.	17	5	0.1	22	0.06	8

FIGURE 2. **A**, merozoite surface protein 1 (MSP-1) (block 2) and MSP-2 (repeats) analysis of consecutive samples collected from four asymptomatic individuals, as indicated in **B** during the 1990 rainy season in Dielmo, Senegal. Individuals 07/06, 10/05, 11/13, and 13/03 were eight, seven, seven, and 26 years old, respectively. **B**, days of blood sampling and corresponding parasite densities. bp = basepairs; individ. = individual; paras. = parasites; n.a. = not available.

lele (700 bp) was detected on days 12, 13, 16–24, and 50. It is interesting to note that on day 14, the three parasite genotypes detected in this individual decreased under the threshold of sensitivity of both PCR and thick smear examination, although they were apparently still present since they became detectable again two days later. In agreement with the results of the first follow-up (Figure 1 and 2), samples collected at 12-day intervals generated different PCR patterns: none of the genotypes amplified on day 1 were still detectable on day 12; similarly on day 50, four previously undetected genotypes were observed, together with two alleles (a 700-bp, IND/3D7-type and an 590-bp, unassigned allele) that had been previously detected.

The results presented in Figure 5 also show fluctuating MSP-1 and MSP-2 patterns. In contrast with the previous examples, most of the genotypes observed in the samples collected between day 12 and day 15 (comprising a total of five distinct MSP-1 alleles and five distinct MSP-2 alleles) were no longer detected thereafter. Other parasite genotypes were detected from day 22 onward, and in addition, a novel MSP-2 allele was amplified from the samples collected from



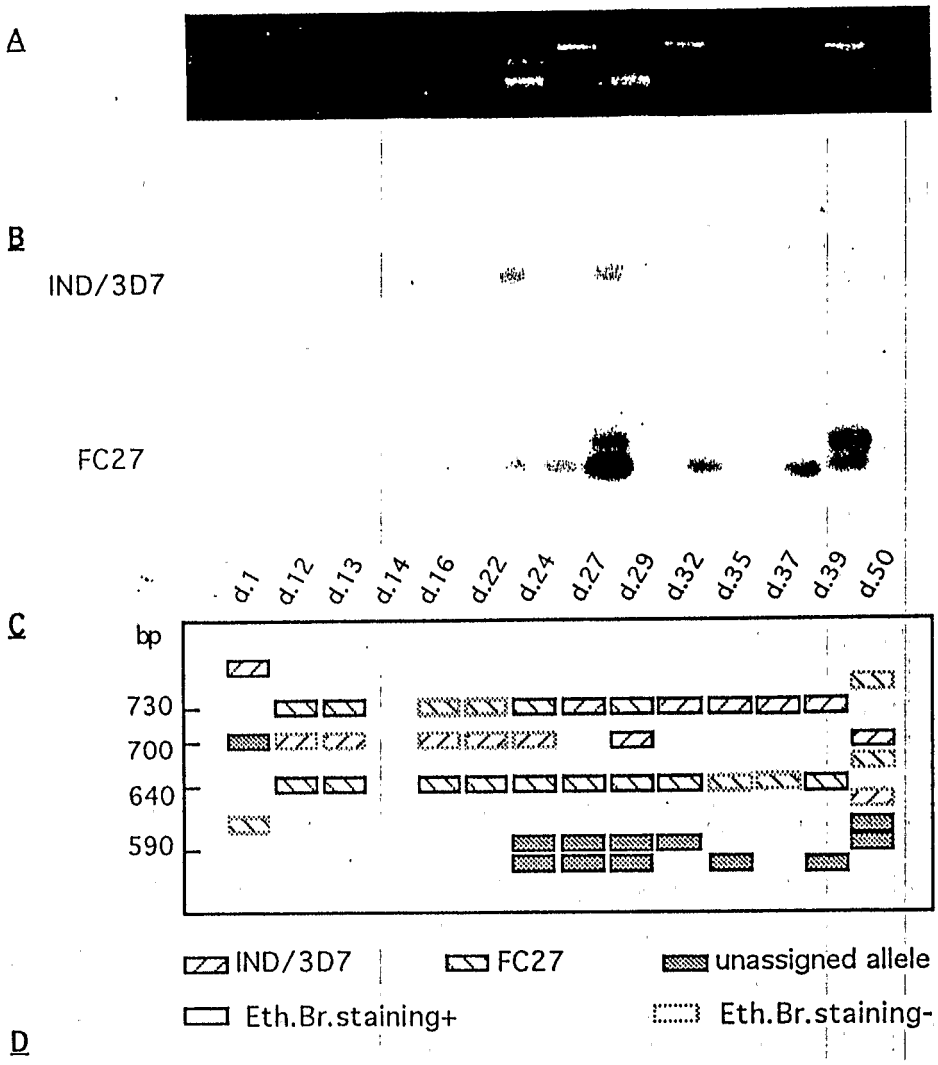
day of sampling	1	2	3	5	6	9	12	14	16	19	21	24
paras./100 leukocytes	3.5	0	4	0.2	0.5	0.5	7.5	14	11	n.a.	n.a.	7

FIGURE 3. Polymerase chain reaction typing of merozoite surface protein 1 (MSP-1) (block 2) alleles in successive blood samples collected from a 52-year-old asymptomatic Dielmo, Senegal villager during the 1992 rainy season (individual code 04/01). **A**, results of hybridization with family-specific probes (K1, MAD20, and RO33) used sequentially on the same filter. **B**, schematic representation of the alleles detected in the various blood samples; codes for each family are as indicated. **C**, days of blood sampling and corresponding parasite densities. bp = basepairs; Eth.Br. = ethidium bromide; paras. = parasites; n.a. = not available. The ethidium bromide–stained bands were of insufficient intensity to be shown here.

days 31–37. Therefore, the patterns observed for this individual reconfirm that in general, there were significant differences between samples collected at 10–15-day intervals. An interesting point was that distinct parasite populations evolve with quite different kinetics. The 580-bp FC27 allele was consistently detected throughout the 50-day survey. On the contrary, other populations apparently decreased rapidly, as indicated by the significantly different patterns observed for both markers in the samples collected two days apart, i.e., on day 13 and day 15.

Analysis of the parasite genotypes in asymptomatic individuals in the absence of transmission: the Pikine study. To evaluate the influence of new parasite inoculations by mosquitoes on the turnover rate of blood parasite populations, a third series of longitudinal studies was carried out in Pikine, a suburb of Dakar, at a time when transmission was interrupted.[9,10] Samples were collected from three teenagers, identified as chronic parasite carriers and recovering, with no symptoms, from a previous malaria attack.

Figure 6 shows the PCR patterns obtained with five samples from each donor, collected every 1–2 weeks, over a total period of five weeks. In contrast to the Dielmo study, a single band was visualized in each sample with both markers, indicating a relatively homogeneous parasite population. With the MSP-1 marker, hybridization with allele-specific probes revealed two very faint bands with the samples from two donors. The patterns remained perfectly stable, showing the same single bands over the five-week survey, with no appearance of any additional amplified bands. The only modification concerned the intensity of the amplifications, which likely reflects the important fluctuations in parasite densities observed microscopically during the survey (Figure

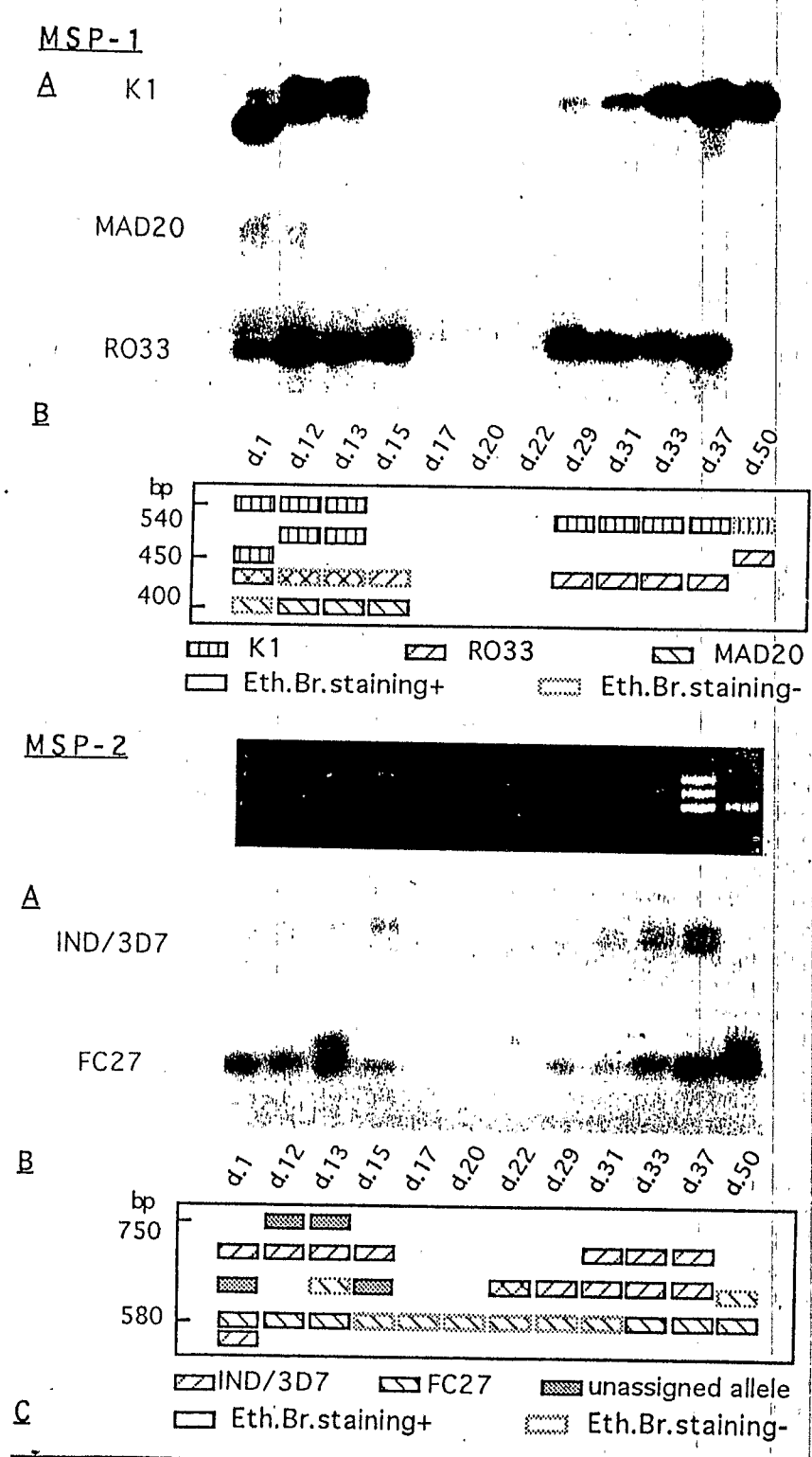


D

day of sampling	1	12	13	14	16	22	24	27	29	32	35	37	39	50
paras./100 leukocytes	0.04	1.5	1.5	0	0.5	4.5	2.5	3	3	n.a.	5	2	28	0.6

FIGURE 4. Polymerase chain reaction (PCR) typing of merozoite surface protein 2 (MSP-2) (repeats) alleles in successive blood samples collected from a 16-year-old asymptomatic Dielmo, Senegal villager during the 1992 rainy season (individual code 18/11). **A,** analysis of the PCR products by ethidium bromide staining of the agarose gel. **B,** results of hybridization with family-specific probes (IND/3D7 and FC27) used sequentially on the same filter. **C,** schematic representation of the alleles detected in the various blood samples; codes for each family are as indicated. **D,** days of blood samples and corresponding parasite densities. For definitions of abbreviations, see Figure 3.

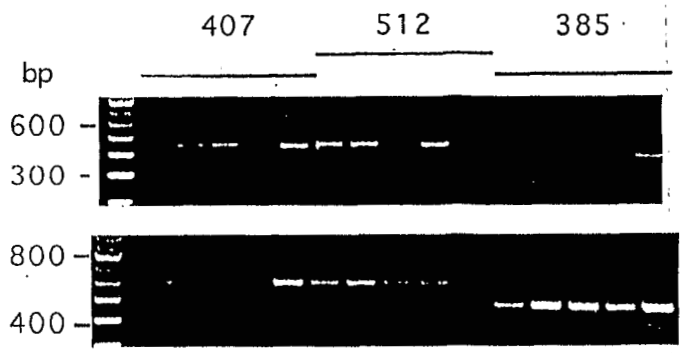
FIGURE 5. Polymerase chain reaction typing of merozoite surface protein 1 (MSP-1) (block 2) and MSP-2 (repeats) alleles in successive blood samples collected from a 49 year-old asymptomatic Dielmo, Senegal villager during the 1992 rainy season (individual code 06/01). **A,** results of hybridization with family-specific probes (K1, MAD20, or RO33 for MSP-1 and IND/3D7 or FC27 for MSP-2) used sequentially on the same filter. **B,** schematic representation of the alleles detected in the various blood samples. Codes for each family are as indicated. **C,** days of blood sampling and corresponding parasite densities. The MSP-1 ethidium bromide–stained bands were of insufficient intensity to be shown here. For definitions of abbreviations, see Figure 3.



C

day of sampling	1	12	13	15	17	20	22	29	31	33	37	50
paras./100 leukocytes	0.7	4	3.5	3.5	0	0	0.5	1	0	n.a.	1	0.25

A



B

day of sampling		1	16	23	30	37
paras./100leuko.	indiv.407	3.12	10	31.2	4	n.a.
	indiv.512	15	15	6.2	13	0.5
	indiv.385	1.56	40	0.12	12.5	99

FIGURE 6. Polymerase chain reaction (PCR) analysis of the parasites (paras.) in successive blood samples collected in Pikine, Senegal from three chronic asymptomatic carriers in January and February 1993, i.e., a period of nontransmission. **A**, analysis of merozoite surface protein 1 (MSP-1) (block 2) and MSP-2 (repeats) PCR products by ethidium bromide staining of agarose gels; the left lane was loaded with a molecular weight marker (100-basepair [bp], ladder). **B**, days of blood sampling and corresponding parasite densities for the three donors. leuko. = leukocytes; indiv. = individual. n.a. = not available.

6B). However, it should be stressed that in contrast to Dielmo, these large variations were not associated with any modification of the PCR patterns, indicating that the same populations were propagated for a long time in the absence of novel inoculations by mosquitoes.

DISCUSSION

In our study conducted in Pikine, we have observed that in the absence of transmission by mosquitoes, PCR patterns obtained with both MSP-1 and MSP-2 primers are simple and remain stable over a period of five weeks. The simplicity of these patterns is consistent with the low endemicity in this region; the low inoculation rate implying that a malaria attack would most likely result from a single infective bite. Furthermore, the three subjects studied here had received a chloroquine treatment resulting in asymptomatic RI-type resistant infections, which can further explain the homogeneity of the parasites harbored. The stability of the PCR patterns was illustrated by the fact that for both genetic markers, no modification of the profiles was observed over a period of five weeks. This was also observed for amplified thrombospondin anonymous protein and circumsporozoite polymorphic regions.[9] Point mutations or minor changes in sequence could not be investigated by the approach used here because this would require DNA sequencing. Nevertheless, our results show that no major allelic changes were observed with these markers, i.e., that the parasites were propagated for a long time without major modification at these loci. This does not rule out the possibility that modifications occurred at other loci, such as those involved in clonal antigenic variation[17,18] and recently identified.[19–21] In very low transmission areas such as Pikine, children and adults do not acquire protection against malaria and are likely to have a clinical episode upon subsequent inoculation. Nevertheless, they are able to support long-term infections by the same parasite. The relationship between strain-specific and strain-independent anti-parasite immunity to clinical immunity in these individuals clearly warrants a detailed investigation.

The results of the longitudinal study performed in Dielmo during the 1990 rainy season showed important and consistent modifications of the PCR patterns with both markers and for all individuals in a time as short as 2–3 weeks, a phenomenon involving both appearance and disappearance of amplification products. Kinetics of parasite turnover were further studied using a closer follow-up conducted during the summer of 1992 on one teenager and two adults. In some cases, PCR patterns changed considerably within only a few days, even though several genotypes could be detected for up to 15 days or even 50 days in one case. Two distinct interpretations can be proposed. First, as stable patterns prevail in the absence of transmission while changing patterns are observed in a period of intense transmission, this rapid turnover may reflect frequent renewal of parasite populations resulting from sporozoite inoculations. In that case, the turnover rate of parasite populations detected in the peripheral blood of Dielmo villagers would indicate that asymptomatic carriage consists of sequential waves of inoculation and partial or total parasite clearance. Since the cumulative exposure

to malaria differs considerably between Dielmo and Pikine inhabitants, the question of whether elimination results from a strain-specific immune mechanism or from competition of distinct strains for the same niche has yet to be resolved. An alternative interpretation would be that the appearance and disappearance of amplification products may essentially reflect fluctuations of the allele ratios,[9] and thus that the observed modifications of PCR patterns result from fluctuations in the relative proportion of distinct parasite populations maintained in long-term infections. Modifications of the density of a specific genotype in the peripheral circulation could be due to strain competition, to strain-specific immune response, and/or to the temporary sequestration in the deep vasculature. These two interpretations of the rapidly changing PCR patterns are not mutually exclusive. Further investigations are needed to determine whether the constant turnover represents successive waves of infection/elimination or whether it corresponds to asynchronous fluctuations of long-term infections in which mosquito inoculations represent a minor contribution.

Our data indicate that there are major quantitative and qualitative variations in the parasite antigen alleles harbored by asymptomatic individuals. In the few cases analyzed, different dynamics were observed and no general picture could be drawn. Indeed, the period over which a specific allele could be detected was highly variable, ranging from a single day to 50 consecutive days. Some alleles were fluctuating concomitantly while others had apparently independent and asynchronous fates. The parasites present in a given asymptomatic individual can therefore be considered as a patchwork of subpopulations that will constantly present different antigens to the immune system.

Another important observation was that the turnover of parasites was as active in adults as in 3–8-year-old children. In Dielmo, as in other holoendemic areas, the mean parasite density is significantly lower in adults than in children less than 15.[7] These children can harbor important parasite loads, while remaining asymptomatic during prolonged periods of time. This is usually interpreted as indicating that the anti-disease immunity develops more rapidly that the anti-parasite immunity.[22] The significant reduction (or even elimination) of many distinct genotypes that we observed here over short periods of time suggests that the children may also possess an efficient anti-parasite immunity. However, children still carry high parasite loads and have a significant probability of experiencing a clinical episode. This suggests that the immune response developed by children provides the possibility to maintain most parasite populations under the threshold of pathogenicity. In the light of our results, it would be important to determine whether this apparently strain-specific anti-parasite response mounted by children is an early manifestation of the phenomenon that will ultimately lead to premunition or whether it constitutes a distinct type of immunity.

Acknowledgments: We are grateful to the Senegalese health authorities and field workers, and especially the inhabitants of Dielmo and Pikine, for kind participation in this project. We also thank Dr. D. Fidock for critically reading the manuscript.

Financial support: This work was supported by grants from the Commission of the European Community Program (no. CT910884), the Ministere de la Cooperation et du Developpement, and the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases.

Authors' addresses: Pierre Daubersies, Selma Sallenave-Sales, Sebastien Magne, and Pierre Druilhe, Laboratory of Biomedical Parasitology, Pasteur Institute, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France. Jean-Francois Trape, ORSTOM, BP 1386, Dakar, Senegal. Hugues Contamin, Thierry Fandeur, and Odile Mercereau-Puijalon, Unit of Experimental Parasitology, Pasteur Institute, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France. Christophe Rogier, Pasteur Institute, BP 220, Dakar, Senegal.

Reprint requests: Pierre Druilhe, Laboratory of Biomedical Parasitology, Pasteur Institute, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France.

REFERENCES

1. Sergent E, Parrot L, 1935. L'immunite, la premunition et la resistance innee. *Arch Inst Pasteur Algeria 13:* 279–319.
2. Bottius E, Guanzirolli A, Trape J-F, Rogier C, Konate L, Druilhe P, 1995. Malaria: even more chronic in nature than previously thought; evidence for submicroscopic parasitemia detectable by PCR. *Trans R Soc Trop Med Hyg:* (in press).
3. Molineaux L, Gramiccia G, 1980. *The Garki Project. Research on the Epidemiology and Control of Malaria in the Sudan Savanna of West Africa.* Geneva: World Health Organization.
4. Babiker HA, Creasey AM, Fenton B, Bayoumi RAL, Arnot DE, Walliker D, 1991. Genetic diversity of *Plasmodium falciparum* in a village in Eastern Sudan. 1. Diversity of enzymes, 2D-PAGE proteins and antigens. *Trans R Soc Trop Med Hyg 85:* 572–577.
5. Conway DJ, Greenwood BM, McBride JS, 1991. The epidemiology of multiple-clone infections in Gambian patients. *Parasitology 103:* 1–6.
6. Mercereau-Puijalon O, Jacquemot C, Sarthou J-L, 1991. A study of the genomic diversity of *Plasmodium falciparum* in Senegal. 1. Typing by Southern blot analysis. *Acta Trop 49:* 281–292.
7. Trape J-F, Rogier C, Konate L, Diagne N, Bouganali H, Canque B, Legros F, Badji A, Ndiaye G, Ndiaye P, Brahimi K, Faye O, Druilhe P, Da Silva LP, 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.
8. Ntoumi F, Contamin H, Rogier C, Bonnefoy S, Trape J-F, Mercereau-Puijalon O, 1995. Age-dependent carriage of multiple *Plasmodium falciparum* MSA-2 alleles in asymptomatic malaria infections. *Am J Trop Med Hyg 52:* 81–88.
9. Contamin H, Fandeur T, Bonnefoy S, Skouri F, Ntoumi F, Mercereau-Puijalon O, 1995. PCR typing of field isolates of *Plasmodium falciparum. J Clin Microbiol 33:* 944–951.
10. Trape J-F, Lefebvre-Zante E, Legros F, Ndiaye G, Bouganali H, Druilhe P, Salem G, 1992. Vector density gradients and the epidemiology of urban malaria in Dakar, Senegal. *Am J Trop Med Hyg 47:* 181–189.
11. Trape J-F, Lefebvre-Zante E, Legros F, Druilhe P, Rogier C, Bouganali H, Salem G, 1993. Malaria morbidity among children exposed to low seasonal transmission in Dakar, Senegal and its implications for malaria control in tropical Africa. *Am J Trop Med Hyg 48:* 748–756.
12. Trape J-F, 1985. Rapid evaluation of malaria parasite density and standardization of thick smear examination for epidemiological investigations. *Trans R Soc Trop Med Hyg 79:* 181–184.
13. Tanabe K, Mackay M, Goman M, Scaife JG, 1987. Allelic dimorphism in a surface antigen gene of the malaria parasite *Plasmodium falciparum. J Mol Biol 195:* 273–287.
14. Smythe JA, Peterson MG, Coppel R, Saul AJ, Kemp DJ, Anders RF, 1990. Structural diversity in the 45-kilodalton merozoite surface antigen of *Plasmodium falciparum. Mol Biochem Parasitol 39:* 227–234.
15. Certa U, Rotmann D, Matile H, Reber-Liske R, 1988. A naturally occurring gene, encoding the major surface antigen pre-

cursor p190 of *Plasmodium falciparum* lacks tripeptide triplets. *EMBO J 6:* 4137–4142.

16. Mercereau-Puijalon O, Fandeur T, Bonnefoy S, Jacquemot C, Sarthou J-L, 1991. A study of the genomic diversity of *Plasmodium falciparum* in Senegal. 2. Typing by use of polymerase chain reaction. *Acta Trop 49:* 293–304.
17. Hommel M, David FH, Oligino LD, 1983. Surface alterations of erythrocytes in *Plasmodium falciparum* malaria. *J Exp Med 157:* 1137–1148.
18. Roberts DJ, Craig AG, Berendt AR, Pinches R, Nash G, Marsh K, Newbold CI, 1992. Rapid switching to multiple antigenic and adhesive phenotypes in malaria. *Nature 357:* 689–692.
19. Baruch DI, Pasloske BL, Singh HB, Bi X, Ma XC, Feldman C, Taraschi TF, Howard RJ, 1995. Cloning the *P. falciparum* gene encoding PfEMP1, a malaria variant antigen and adherence receptor on the surface of parasitized human erythrocytes. *Cell 82:* 77–87.
20. Su X-Z, Heatwole VM, Wertheimer SP, Guinet F, Herrfeldt JA, Peterson DS, Ravetch JA, Wellems TE, 1995. The large diverse gene family *var* encodes proteins involved in cytoadherence and antigenic variation of *Plasmodium falciparum*-infected erythrocytes. *Cell 82:* 89-100.
21. Smith JD, Chitnis CE, Craig AG, Roberts DJ, Hudson-Taylor DE, Peterson DS, Pinches R, Newbold CI, Miller LH, 1995. Switches in expression of *Plasmodium falciparum var* genes correlates with changes in antigenic and cytoadherent phenotypes of infected erythrocytes. *Cell 82:* 101–110.
22. Rogier C, Trape J-F, 1993. Malaria attacks in children exposed to high transmission: who is protected? *Trans R Soc Trop Med Hyg 87:* 245–246.