Genetic Characterization of an Epidemic of *Plasmodium falciparum* Malaria among Yanomami Amerindians

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Malaria parasites are genetically diverse at all levels of endemicity. In contrast, the merozoite surface protein (MSP) alleles in samples from 2 isolated populations of Yanomami Amerindians during an epidemic of *Plasmodium falciparum* were identical. The nonvariable restriction fragment length polymorphism patterns further suggested that the sequential outbreak comprised only a single *P. falciparum* genotype. By examination of serial samples from single human infections, the MSP characteristics were found to remain constant throughout the course of infection. An apparent clonal population structure of parasites seemed to cause outbreaks in small isolated villages. The use of standard molecular epidemiologic methods to measure genetic diversity in malaria revealed the occurrence of a genetically monomorphic population of *P. falciparum* within a human community.

*Plasmodium falciparum* parasites are genetically diverse in all areas where malaria transmission is stable [1, 2] and even in areas of low endemicity where transmission is unstable [3]. These observations have prompted some investigators to propose that in the natural setting, populations of malaria parasites are not clonal but result from cross-fertilization of multiple genomic types [4]. It may be possible for malaria parasites to be genetically monomorphic where transmission is infrequent and the human population is isolated, as among the traditional hunter-gatherer residents of the Americas. To evaluate this hypothesis, we measured genetic diversity among *P. falciparum* parasites sampled from 2 isolated villages of Yanomami Amerindians (Coyoweteri and Matoweteri) that were experiencing sequential outbreaks of *P. falciparum* malaria [5]. Genetic diversity was evaluated by standard molecular epidemiologic methods examining the polymorphic regions of the merozoite surface protein (MSP)-1 and MSP-2 genes [1–3]. These outbreaks presented a unique field opportunity to genetically characterize an epidemic of *P. falciparum* in a defined community.

**Methods**

**Study population.** The study population consisted of all residents of 2 Yanomami villages in Amazonas State, Venezuela, who took part in a prospective malaria survey conducted from August 1993 to November 1994 [5]. The more accessible of these villages, Coyoweteri (designated the Mission Village), included 62 Yanomami residents and members of several missionary families who did not participate in the study. The other village, Matoweteri (designated the Remote Village), included 70 residents and was located 5 days by foot from the Mission Village. The 132 Amerindians who participated in the study comprised the entire Yanomami population of each village, as best could be determined, during the time of 2 sequential outbreaks of *P. falciparum*, in October and November 1994. Blood was sampled from all residents of both villages, whether or not they had symptoms (designated as outbreak samples). After the outbreak samples were obtained, antimalarial treatment was administered to all persons infected with *Plasmodium* parasites.
A series of comparison villages was designated for comparison with the villages involved in the 2 sequential outbreaks. These included the Yanomami villages of Obowacateri, Parima B, Coshikoweteri, and the indigenous communities of Guahibo Amerindians near Puerto Ayacucho. Samples were obtained from the Yanomami villages from August 1993 through September 1994 [5] and from the Guahibo communities in January 1992 [6].

Sample processing and polymerase chain reaction (PCR) analysis. DNA was amplified by PCR from each blood sample, as described elsewhere [6]. For comparison, 5–50 ng of purified genomic DNA from a laboratory strain (W2 from Indochina [7]) was suspended in 100 μL of the same PCR reaction mixture. Oligonucleotide primers to the MSP-1 and MSP-2 genes were synthesized (Biosearch 8700 DNA synthesizer; New Brunswick Scientific, Edison, NJ). Primers to the MSP-1 gene were derived from the conserved flanking regions of the block 2 repeat region [8]. Primers to the MSP-2 gene were derived from the conserved regions flanking the central variable region [8]. Blood samples were amplified for 40 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 45 s [8].

Agarose gel electrophoresis and Southern blot analysis. Amplified regions of MSP-1 and MSP-2 alleles were analyzed by standard gel electrophoresis (2.5% 3:1 agarose for small DNA, 1× TAE [Tris, sodium acetate, and EDTA], 70 V). MSP-1 and MSP-2 band sizes were measured from a standard curve incorporating *PhiX174/HaeIII*. Products that were not visualized in this manner were subjected to Southern blotting and hybridized with 32P-labeled MSP-1 or MSP-2 PCR product from a representative patient sample used as a radioactive probe. The probe was labeled with 32P by the random oligo method of incorporation [9].

Restriction fragment length polymorphism (RFLP) analysis. The MSP-1 PCR products of all amplified samples were digested by use of the restriction enzyme *SacI* (New England Biolabs, Beverly, MA). After digestion, restriction digest products were visualized on standard gel electrophoresis or through Southern blot and hybridization, as described above. The same radioactive probe was used for both the full MSP-1 product and the restriction products.

**Results**

During the outbreaks in October and November 1994, there were 32 cases of *P. falciparum* among the 70 Remote Village inhabitants and 28 cases among the 62 Mission Village residents (60 infections in 132 blood samples). In contrast, earlier cross-sectional surveys of the 2 villages, in August and September 1993 and May–July 1994, identified only 14 *P. falciparum* infections among 320 blood samples, demonstrating low endemicity [5]. This outbreak represented a significant increase over previous levels of infection ($\chi^2 = 115.18$, 1 df; $P < .001$). Of the 60 malaria infections observed in the epidemic, 47 were symptomatic, and 3 persons were in coma when brought to the health post in the Mission Village.

In early October, when residents of the Remote Village became ill, the entire population traveled by foot for 5 days to the Mission Village in search of medical attention. About 3 weeks after their arrival, the Mission Village experienced a marked increase in cases of *P. falciparum* (figure 1). The steep rise in infections in both villages suggested that each outbreak arose from a single source. Samples were available from 54 of the 60 residents infected during the outbreak, and these were used as templates for PCR amplification. Of these, 37 amplified with both sets of primers, 6 amplified solely with the MSP-1 set of primers, and 11 did not amplify with either primer set. These nonamplified samples generally had low parasite density and were distributed evenly between the 2 villages and by age.
and gender (data not shown). We visualized electrophoretically 26 of the 43 amplified MSP-1 samples and 17 of the 37 amplified MSP-2 samples. All fragments of the visualized MSP-1 samples were 580 bp long, and all fragments of the MSP-2 samples were 780 bp long (figure 2A, 2B). These fragments differed in length from those of a standard laboratory strain (W2, Indochina: 620 bp for MSP-1 and 820 bp for MSP-2). Of the samples that could be visualized electrophoretically, the molecular weights of the MSP genes appeared to be identical.

DNA hybridization, used to detect polymorphisms in the samples that could not be visualized by electrophoresis, identified MSP-1 gene fragments in all 17 samples analyzed and MSP-2 gene fragments in all 20 samples analyzed. As in the case of samples analyzed solely by electrophoresis, fragments of the MSP-1 gene were 580 bp long, and those of the MSP-2 gene were 780 bp long. Thus, the MSP genes of all *P. falciparum* samples in the sequential outbreaks appeared to be identical, regardless of the technique used for analysis.

MSP-1 amplification products of all samples were then subjected to RFLP analysis as an independent measure of monomorphism. Only 1 fragment pattern resulted from *Rsa* I restriction enzyme digestion, and this pattern was distinct from that produced with W2 DNA (figure 2C). These nonvariable RFLP patterns suggest that the sequential outbreaks comprised only a single *P. falciparum* genotype.

Because all samples from the sequential outbreaks were taken early during the course of infection, we considered the possibility that different genotypes might emerge later during the same infection. Thus, we examined MSP-1 amplification products sampled sequentially from an individual resident of each village. All these samples appeared to be identical, indicating that *P. falciparum* MSP characteristics remained constant throughout the course of infection (data not shown).

To determine the genetic diversity of *P. falciparum* in this region of the Venezuelan Amazon, samples were obtained from comparison Yanomami villages [5] and from Guahibo Amerindian communities [6]. Unlike the monomorphic parasite populations in the outbreak, other parasite populations in this region were genetically diverse (data not shown).

**Discussion**

Here we describe an epidemic of *P. falciparum* among the Yanomami. We used molecular techniques to describe the genetic composition of the parasites involved. Two outbreaks of *P. falciparum* malaria represented a significant increase in prevalence over that observed at other survey points, even accounting for expected seasonal variation of malaria in this region. Movement between these 2 villages seemed to be linked to these outbreaks. By standard examination of the genes for MSP-1 and MSP-2, we demonstrated a single strain of parasites involved in the epidemic for both villages, in contrast with the genetic diversity previously observed in this region and in other areas of the world studied by the same techniques. These findings shed light on the microepidemiology of malaria among remote and isolated populations.

Cultural habits of the Yanomami may explain this unique occurrence of an epidemic due to a single strain of *P. falciparum* [5]. Contact with other villages is limited, and a lifestyle that
results in little disruption of the environment may minimize exposure to Anopheles mosquitoes. However, Yanomami villages periodically travel as a single unit, including during times in which they attempt to flee from a disease outbreak and seek medical attention. During such travels, 1 village can introduce infection into another. Because Yanomami villages consist of up to 200 persons living in 1 single-roomed house (shabono), an epidemic can rapidly spread throughout the entire village. The source of the single genotype that produced this epidemic may have been a single infected person. Missionaries in the area reported that a P. falciparum-infected Yanomami boy visited Coyoweteri late in August. He failed to complete a recommended course of treatment but returned to his home in Matoweteri where he died soon thereafter.

The geographic isolation of the villages may have impeded introduction of new populations of parasites, thus accounting for the presence of only 1 genotype [4]. Alternatively, the presence of a single genotype also may have resulted from a unique ability to survive the dry season, resist antimalarial drugs, or evade strain-specific acquired immunity [10]. The persistence of a single genotype in a population supports the hypothesis of clonal propagation of malaria parasites, as proposed by Tibayrenc et al. [4], or a high degree of self-fertilization, as discussed by Day et al. [2].

We considered the possibility that our study design, which relied exclusively on samples taken on the day that subjects first reported for treatment, failed to include the full range of diversity present in P. falciparum infections present in the region. A sequence of genotypes might emerge over the course of each infection [11, 12]. Our sequential analysis confirmed other reports of the genetic stability of a single genotype throughout infection [11, 13].

DNA sequence data was not obtained in this study, limiting our ability to confirm a single MSP-1 and MSP-2 genotype. Agarose gel analysis is limited in that PCR amplicons of differing DNA sequences with the same molecular weight will appear to be identical, and size differences below the resolution of the gel cannot be determined. Results from the more sensitive restriction digests, however, strongly suggested that patient samples comprised a single genotype. Although monomorphism in MSP-1 and MSP-2 genes does not imply monomorphism in other genes, this study contrasts the tremendous genetic diversity typically observed in MSP-1 and MSP-2 genes [1–3, 12, 13].

In other studies that used similar molecular techniques, P. falciparum infections were found to differ genetically, even within a single household, and individuals were found to harbor multiple genotypes [12–14]. Diversity might be expected to correlate with transmission intensity, because human superinfection would result in superimposition of each of the genotypes carried by different vector mosquitoes [3]. Diversity would also correlate with human movement; thus, individual residents of a highly endemic village, such as those in sub-Saharan Africa, might thereby rapidly receive numerous sporozoite inoculations derived from a large catchment area. In such areas, recombination events in the Anopheles vector would produce novel genotypes [15]. In contrast, our results suggest that single genotype populations may persist, in particular among geographically isolated populations in areas of low endemicity. Our findings may represent an extreme example of the direct relationship between the amount of genetic diversity of malaria populations and the transmission intensity in a particular area [3].

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References

