RESTRICTED GENETIC DIVERSITY OF PLASMODIUM FALCIPARUM MAJOR MEROZOITE SURFACE PROTEIN 1 IN ISOLATES FROM COLOMBIA

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Abstract. The merozoite surface protein 1 (MSP-1) gene of Plasmodium falciparum encodes a major immune target under development as a malaria vaccine. In this study, we typed MSP-1 variable regions of parasites obtained from Buenaventura, Colombia. Four MSP-1 gene types were detected corresponding to prototype and recombinant K1 and MAD20 block 4 sequences. In contrast to variability within block 4, blocks 2, 6, and 16–17 corresponded exclusively to the MAD20 allelic type. Most (80%) blood samples contained multiple MSP-1 gene types. The presence of four MSP-1 variants within block 4 against a MAD20 background indicates that current P. falciparum populations in Buenaventura are derived from parasites expressing K1 and MAD20 alleles, some of which underwent two recombination events within or flanking block 4. Restricted MSP-1 diversity appears to be relatively stable in Buenaventura and suggests that selection has resulted in the dominance of the MAD20 type in most of the polymorphic blocks with the exception of block 4.

INTRODUCTION

The major merozoite surface protein 1 (MSP-1) of Plasmodium falciparum undergoes processing during formation and maturation of merozoites to form four major fragments of approximately 83, 28–30, 38, and 42 kD. The 42-kD polypeptide is further cleaved into 33- and 19-kD fragments and the latter remains at the surface of the ring-stage parasite after invasion of the host erythrocyte. The MSP-1 gene can be divided into 17 sequence blocks that are either conserved, semi-conserved, or variable. The MSP-1 gene is dimorphic at each block for either K1 or MAD 20 with the exception of block 2, which has an additional allele RO33, and block 4, which may either be of the K1 or MAD20 prototypes or a recombinant of these types. Thus, the MSP-1 gene can have the following combinations: one of three versions of block two: K1, MAD20, or RO33; one of four possibilities of block 4: MM, KK, MK, or KM; and one of two possibilities for block 6 through 16: K1 or MAD20. These combinations present 24 possible gene types for MSP-1 and sequence variation occurs in more than 60% of the gene. Antigenic polymorphism of MSP-1 corresponding to this sequence variability has been demonstrated using a panel of variant-specific monoclonal antibodies. A polymerase chain reaction (PCR) strategy was developed to determine the 24 different MSP-1 association types based on block 2, 4a, 4b, and 10–17 to investigate allelic variation in the MSP-1 gene. Their study from southern Vietnam showed 1) that recombination between two representative allelic types in the central part of the MSP-1 gene did not exist, 2) the frequency distribution of MSP-1 association types did not differ in different population groups, and 3) particular MSP-1 association types were predominant. Other studies of MSP-1 genetic diversity in Brazil have suggested that selection operates in favor of particular MSP-1 association types.

Antigenic polymorphism is a potential limitation for developing a P. falciparum MSP-1–based malaria vaccine. In contrast to the N-terminal region of MSP-1, a limited degree of polymorphism has been documented for the 19-kD C-terminal region, which has been attributed to point mutation and recombination. In this study, we have analyzed the genetic diversity in the N-terminal region (blocks 2, 4, and 6) and the C-terminal region (block 16) of MSP-1 using PCR on DNA obtained from parasites of children and adults infected with P. falciparum in the malaria-endemic region of Buenaventura, Colombia.

MATERIALS AND METHODS

Study site. The study was conducted in Buenaventura, a port city located on the Pacific coast of Colombia. It has 292,373 inhabitants, 85.6% of whom live in the urban area. The population of the four coastal states located along the Pacific coast is nearly one million (2.5% of the national population) and the malaria cases of this region represent 12.5% of the total cases in the country. Colombia reported 206,195 malaria cases in 2001, and 93,633 of these were due to infection with P. falciparum. In Buenaventura, 16,578 malaria cases were reported in 1998 and 10,238 in 2001. Malaria transmission is low and unstable in this disease-endemic area and Anopheles albimanus is the primary vector in the urban area. Anopheles neivai and An. nuneztovari are common in the rural area. Buenaventura is characterized by relatively high humidity (80–90%) and receives 8,000 mm of annual rainfall. The average temperature is 26°C (78.8°F).

Plasmodium falciparum isolates. Blood samples positive for P. falciparum were collected from symptomatic patients (patients with fever and headache) who attended the Program for Tropical Diseases and Immunology Institute in Buenaventura after consent was provided to Malaria Vaccine and Drug Development Center health personnel. The study protocol was reviewed and approved by the Institutional Review Board of Universidad del Valle. Patients attending the health center came from Buenaventura (patient population = 270,000) and the surrounding rural area (patient population = 4,331) and provide a representative sample of malaria cases in the rural and urban areas. Forty-six blood samples were obtained at three different times: 18 samples (01–20) were collected in February 1998, 11 samples (Z01–Z12) were collected in October 1998, and 17 samples (1F–17F) were collected in February 1998. The following combinations: one of three versions of block two: K1, MAD20, or RO33; one of four possibilities of block 4: MM, KK, MK, or KM; and one of two possibilities for block 6 through 16: K1 or MAD20. These combinations present 24 possible gene types for MSP-1 and sequence variation occurs in more than 60% of the gene. Antigenic polymorphism of MSP-1 corresponding to this sequence variability has been demonstrated using a panel of variant-specific monoclonal antibodies. A polymerase chain reaction (PCR) strategy was developed to determine the 24 different MSP-1 association types based on block 2, 4a, 4b, and 10–17 to investigate allelic variation in the MSP-1 gene. Their study from southern Vietnam showed 1) that recombination between two representative allelic types in the central part of the MSP-1 gene did not exist, 2) the frequency distribution of MSP-1 association types did not differ in different population groups, and 3) particular MSP-1 association types were predominant. Other studies of MSP-1 genetic diversity in Brazil have suggested that selection operates in favor of particular MSP-1 association types.

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collected in July 2001. *Plasmodium falciparum* parasitemia in these samples varied from 5 to 350 parasites/100 white blood cells. Each sample was identified by patient origin, age, and sex, and parasitemia was determined by a thick blood smear stained with Giemsa.

**Isolation of *P. falciparum* DNA from blood of infected humans.** Blood (0.5–1 mL) was withdrawn from each patient and 500 μL of lysis buffer (1.6 M sucrose, 5% [v/v] Triton X-100, 25 mM MgCl₂, 60 mM Tris-HCl, pH 7.5) was added to the blood. The mixture was centrifuged at 13,000 rpm for one minute in an Eppendorf (Hamburg, Germany) microcentrifuge. The supernatant was discarded and the excess liquid was air-dried. The pellet was resuspended in 5x filtered proteinase K buffer (0.375 M NaCl, 0.12 M EDTA, pH 8), proteinase K, and 20% sodium dodecyl sulfate, and adjusted up to a volume of 400 μL with distilled water. The mixture was incubated at 55°C with constant shaking for at least 30 minutes. One volume of phenol-chloroform was then added and mixed by vortexing to remove protein contaminants. The sample was then centrifuged at 10,000 rpm for five minutes at room temperature. The aqueous phase was transferred to a new vial, precipitated with cold ethanol, and incubated at −70°C for one hour. The vial was centrifuged at 13,000 rpm for 15 minutes at 4°C. The DNA was pelleted by centrifugation at 13,000 rpm for five minutes and the pellet was washed with 70% ethanol. The supernatant was discarded and the DNA pellet was washed again with 70% ethanol. The sample was centrifuged at 13,000 rpm for five minutes and the DNA pellet was dried. Gene Clean III kit (Bio 101, Irvine, CA) was used to further purify the isolated DNA.

**Plasmodium falciparum** MSP-1 *typing of blocks 2, 4a, 4b, 6, and 16.* Specific PCR primer pairs⁴⁻⁶,⁹ (Gibco-BRL, Gaithersburg, MD) were used to type the different variable blocks of MSP-1 and are shown in Table 1. The location of each of the primers along the MSP-1 gene is shown in Figure 1. Genotyping focused on blocks 2, 4, 6, and 16–17 because although blocks 2 and 4 are variable and block 4 is known to undergo intragenic recombination, blocks 6–16 are concordant in all parasites examined to date.¹⁶ Prototype MSP-1 allelic sequences were derived from the K1, MAD20, and RO33 isolates of *P. falciparum.* The sensitivity and specificity of each primer pair was established using positive control DNA templates obtained from parasites containing each allelic sequence.

**Amplification by PCR.** The PCR mixtures (25 μL) contained 23 μL of PCR Super Mix (Gibco-BRL), 0.6 μL of DNA template, and 0.5 μL of each primer. For amplification of blocks 2 and 16, 50 cycles at 95°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds were used. To type block 4, blocks 3–5 were amplified and a nested PCR was performed using 0.5 μL of the blocks 3–5 PCR products per 25-μL PCR. For these reactions, 25 cycles at 95°C for 30 seconds, 56°C for 30 seconds, and 72°C for 10 seconds were used to amplify the DNA. For block 6, 50 cycles at 95°C for 30 seconds, 56°C for 10 seconds, and 72°C for 10 seconds were used. Every PCR assay included a tube containing no template as a negative control.

**Gel electrophoresis.** The PCR products were subjected to electrophoresis on 2% agarose gels (Gibco-BRL) in Tris-acetate-EDTA buffer for various lengths of time depending of the predicted size of the PCR products and visualized with ethidium bromide by transillumination with ultraviolet light. We used 100 and 50 basepair ladders (Pharmacia Biotech, Piscataway, NJ) as standards for estimation of the sizes of DNA fragments.

**Determination of *P. falciparum* MSP-1 gene type by PCR.** Table 1 shows the set of PCR primers designated to identify 24 distinct MSP-1 gene types targeting sequences in blocks 2, 3–5, 4a, 4b, 6, and 16–17. Typing was done using the PCR primers shown in Table 2 and based on the predicted sizes of PCR products. Our strategy to determine MSP-1 gene type was as follows. First, the allelic type of block 2 was determined by PCR in three separate reaction mixtures containing any of three allele-specific primers (K2F, M2F, or R2F) and the reverse primer C3R. Second, block 3–5 was amplified with C3F as forward primer and C3R as reverse primer. Third, the allelic type of block 4 (4a/4b) was determined in four different reactions of the block 3–5 PCR product with all combinations of the M4AF, K4AF forward primers and M4PR and K4PR reverse primers.

**Data analysis.** Variation in haplotype frequencies between populations from different geographic areas was analyzed using Wright's *F*⁶ statistics as computed by the Arlequin version 2.000 population genetics software program (Genetics

### Table 1

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequences 5' → 3'</th>
<th>Specificity</th>
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<td>TCTTAAATGAGAAGAATTACTAAAAA</td>
<td>K1 block 2</td>
</tr>
<tr>
<td>M2F</td>
<td>GGTTCCAGTATTCAAGGCTAC</td>
<td>MAD20 block 2</td>
</tr>
<tr>
<td>R2F</td>
<td>TAAAGGATGGAGCAAATACCAAGT</td>
<td>RO33 block 2</td>
</tr>
<tr>
<td>C3F</td>
<td>AGATATGATGGTGAATATCAAAGAG</td>
<td>Common block 3</td>
</tr>
<tr>
<td>C3R</td>
<td>TTCTGGAATAATGAAATTAGCGTAC</td>
<td>Common block 3</td>
</tr>
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<td>K4AF</td>
<td>AATGAAATTTAATCCCTACCGG</td>
<td>K1 block 4a</td>
</tr>
<tr>
<td>M4AF</td>
<td>TTGGAGATATTAGATAATTAAATACAGTG</td>
<td>MAD20 block 4a</td>
</tr>
<tr>
<td>K4PR</td>
<td>TCGACTTCTTTTTTTTGCTTATCAAG</td>
<td>K1 block 4b</td>
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<tr>
<td>M4PR</td>
<td>TCAGACTTCTTTTTTTTGCTTATCAAG</td>
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<td>C5F</td>
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<td>Common block 5</td>
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<tr>
<td>C5R</td>
<td>GATGATGGTTTTTCTTATTCTCAG</td>
<td>Common block 5</td>
</tr>
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<td>K6R</td>
<td>GATATTTTTTTTCTTATTCTCAG</td>
<td>K1 block 6</td>
</tr>
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<td>M6R</td>
<td>ATTTGGAACAGATTTGGATGTCCTG</td>
<td>MAD20 block 6</td>
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<td>K16F</td>
<td>CGGTTTTATCTAATTCTCTGATTGAA</td>
<td>K1 block 16</td>
</tr>
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<td>M16F</td>
<td>CCTAATAAATAATAATCAAATATTGA</td>
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</tr>
<tr>
<td>C3FIR</td>
<td>ATTAAGGTAACATATTTTAACTTAC</td>
<td>Common block 16/17</td>
</tr>
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and Biometry Laboratory, University of Geneva, Geneva, Switzerland). The MSP1 gene diversity (H) in different populations was estimated as

\[ H = \frac{n}{n-1} \left( 1 - \sum_{i=1}^{k} p_i^2 \right) \]

where \( n \) is the number of isolates sampled, \( k \) is the number of haplotypes, and \( p_i \) is the sample frequency of the \( i \)th haplotype using Arlequin version 2.000.

**RESULTS**

The PCR amplification of the variable regions of *P. falciparum* MSP-1 was performed on 46 blood samples collected in 1998 and 2001 from malaria patients in Buenaventura, Colombia (Table 3). Of the subjects analyzed, 25 (54%) were female and 20 (46%) were male. The blood samples used in this study were from 3 children ≤ 10 years of age (one 8 years old and two 10 years old, 6.52%), 17 children and young adults between 11 and 20 years of age (37%), 11 adults between 21 and 30 years of age (24%), and 15 adults between 31 and 50 years of age (32.48%). Forty-four of the 46 patient samples (96%) were successfully characterized for MSP-1 genotype by PCR. The MSP-1 allelic types for blocks 2, 6, and 16 corresponded exclusively to the M allele for all three sampling times. No size variation was noted for PCR products containing the block 2 variable repeat region in these samples. In contrast, MSP-1 block 4 showed substantial variation with sequences corresponding to both prototypic (K and M) and recombinant (K:M and M:K) allelic types.

Detection of more than one allelic type for block 4 provided evidence for multiple *P. falciparum* infections of many individuals. Based on block 4 typing, nine (20.5%) of the samples were found to have single infections, while 35 (79.5%) of the samples had multiple infections ranging from 2 to 4 distinguishable parasite genotypes. A total of 109 clones were identified among the 46 *P. falciparum* samples by PCR. Frequency distributions of the MSP-1 gene types detected in this study are shown in Figure 2. There was no difference in the sex distribution of the observed gene types. When gene type frequencies were examined for each collection date, some differences were noted (Figure 2). There was a significant difference in the proportion of each allelic type among samples collected during February 1998 (\( P = 0.01 \) by Fisher’s exact test) and July 1998 (\( P = 0.0009 \) by Fisher’s exact test), with a higher representation of types 17 and 20 than types 14 and 23. During October 2001, allelic frequencies were also unequally distributed (\( \chi^2 = 4.86, P < 0.05 \)), but in these samples gene types 14 and 23 were more prevalent than at earlier sampling dates. When the gene type frequencies of pairs of population samples were compared using conven-

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**Table 2**

<table>
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<tr>
<th>Block</th>
<th>Allelic type*</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Fragment size (basepairs)</th>
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<td>K</td>
<td>K2F</td>
<td>C3R</td>
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<tr>
<td></td>
<td>M</td>
<td>M2F</td>
<td>C3R</td>
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<tr>
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<td>R</td>
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<td>C3R</td>
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<tr>
<td>4a–4b</td>
<td>K:K</td>
<td>K4AF</td>
<td>K4PR</td>
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</tr>
<tr>
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<td>K:M</td>
<td>K4AF</td>
<td>M4PR</td>
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</tr>
<tr>
<td></td>
<td>M:K</td>
<td>M4AF</td>
<td>K4PR</td>
<td>94</td>
</tr>
<tr>
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<td>M:M</td>
<td>M4AF</td>
<td>M4PR</td>
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<td>K</td>
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<td>K</td>
<td>K16</td>
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<td></td>
<td>M</td>
<td>M16F</td>
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<td>428</td>
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</table>

* K = K1; M = MAD20; R = RO33.

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**Figure 1.**

a. Basic structure of the *Plasmodium falciparum* merozoite surface protein 1 gene. Conserved, semi-conserved, and variable blocks of the gene are shown as open, hatched, and closed boxes, respectively.

b. Scheme for locations and directions of the oligonucleotide primers used to type variable blocks 2, 4, 6, and 16.
tional F-statistics, $F_{ST}$ values were significantly different for the July 1998 and October–November 2001 samples ($F_{ST} < 0.0005$), while other pairwise comparisons were not significantly different. However, despite variations in gene type frequencies, the same four gene types were detected for all three sampling dates in Buenaventura.

The MSP-1 genotyping results obtained in Buenaventura were compared with those of similar studies carried out in Vietnam, the southwestern Brazilian Amazon, and northern Tanzania. The percentage of isolates with more than one MSP-1 gene type was higher in samples from Buenaventura (80%) than reported for Brazil, Vietnam, or Tanzania (Table 4). The MSP-1 gene diversity ($H^*$) was lower in Buenaventura, an area hypoendemic for *P. falciparum* malaria, than in the Brazilian Amazon, another hypoendemic area, Vietnam (a mesoendemic area), and northern Tanzania (a holoendemic area) (Table 4).

A pairwise comparison of MSP-1 gene type frequencies for the various geographic regions by F statistics indicated that each of these regions were significantly different from each other ($F_{ST} P < 0.001$), with the exception of Brazil and Tanzania (Table 4). The four MSP1 gene types observed in Buenaventura represented 17–58% of the isolates identified in the three other regions. However, gene types 13–24 containing MAD20 allelic types downstream of block 4 predominated in all four geographic areas.

### DISCUSSION

A restricted number of MSP-1 gene types (4 of the 24 possible gene types) were identified in *P. falciparum* samples...
collected in Buenaventura, Colombia in 1998 and 2001. In samples taken from 46 malaria cases, only the MAD20 (M) allelic type was observed for blocks 2, 6, and 16 and no size variation was noted for block 2 containing the variable repeat region. In contrast, block 4 showed remarkable variability, consisting of all four possible MSP1 parental (K1 and MAD20) and recombinant (K:M and M:K) allelic types. Although the proportion of MSP-1 gene types corresponding to the different block 4 allelic types varied somewhat over time, limitation of the parasite population to the same four MSP-1 gene types was stable over the four-year span of the study.

The presence of this pattern of diversity within block 4

<table>
<thead>
<tr>
<th>MSP1 gene type</th>
<th>Vietnam</th>
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<th>Northern Tanzania</th>
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<td>(mesoendemic)</td>
<td>(hypoendemic)</td>
<td>(hypoendemic)</td>
<td>(holoendemic)</td>
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<td>n</td>
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<td>109</td>
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No. gene types detected 19 4 10 13
Percent with >1 gene type 46 80 39 60
Average no. gene types/sample 1.78 2.4 1.42 2.37
Gene diversity (H) 0.8809 ± 0.0118 0.745 ± 0.0118 0.8178 ± 0.0264 0.8661 ± 0.001

<table>
<thead>
<tr>
<th>Population pairwise comparison</th>
<th>Pairwise F-statistics</th>
<th>F-ST</th>
<th>P</th>
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<td>Vietnam vs Colombia</td>
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<td>Vietnam vs Brazil</td>
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<td>≤0.001</td>
<td></td>
</tr>
<tr>
<td>Vietnam vs Tanzania</td>
<td>0.05178</td>
<td>≤0.001</td>
<td></td>
</tr>
<tr>
<td>Colombia vs Brazil</td>
<td>0.18583</td>
<td>≤0.001</td>
<td></td>
</tr>
<tr>
<td>Colombia vs Tanzania</td>
<td>0.12591</td>
<td>≤0.001</td>
<td></td>
</tr>
<tr>
<td>Brazil vs Tanzania</td>
<td>0.00813</td>
<td>0.063</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Frequency distribution of Plasmodium falciparum merozoite surface protein 1 (MSP-1) gene types (shown as bars for types 14, 17, 20, and 23) in 44 patient blood samples collected in February 1998, July 1998, and October–November 2001 in Buenaventura, Colombia. Numbers above bars correspond to number of samples with each gene type.
suggests that parasites currently present in Buenaventura are
descendant from both K1 and MAD20 parasite populations. 
Parasites with MSP-1 allelic types 14, 17, and 20 would have
undergone two recombination events within block 4, between
blocks 3 and 4, and/or between blocks 4 and 5 to generate the
population of recombinant parasites present in this region. 
These recombination events could have occurred prior to or
following introduction to this geographic region. The high
frequency of mixed parasite infections observed in samples
collected in Buenaventura (80%) would increase the poten-
tial for meiotic recombination at the MSP-1 locus in the mos-
quito vector. Similar recombination within MSP1 block 4 and
coeexistence of the four block 4 allelic types were observed in
isolates obtained in the Mae Sod District of Tak Province in
Thailand, in the Solomon Islands, and in the Brazilian Ama-
zon.6,17 Although MSP-1 gene type 20 (block 4 allelic type
K:M) was common among Buenaventura isolates, it was not
detected in the Brazilian Amazon or in Tanzania. The signifi-
cance of sequence diversity within block 4 is unknown. Anti-
body reactivity of block 2 variant peptides by malaria pa-
tients has been described.18,19

Human serologic reactivity with block 4 sequences has not
been evaluated; however, murine monoclonal antibodies spe-
cific for K1 and MAD20 allelic epitopes within block 4 have
been described.20 Thus, it will be of interest to determine
whether humans develop an antibody response to block 4
epitopes and, if so, whether these antibodies discriminate
among the prototypic K and M and chimeric M:K and K:M
allelic types. Alternatively, Block 4 sequences may corre-
spond to T cell epitopes that elicit the activation of type/major
histocompatibility complex–specific CD4+ T helper cells. Di-
 morphic T cell epitopes of P. falciparum MSP-1 that overlap
with the N-terminal border of block 4, which are frequently
recognized by interferon-γ (Th1) and interleukin-4 (Th2) and
produce T cells of east and west African donors, have been
described.9,21

Previous studies of MSP-1 allelic types in Colombia have
provided varying patterns of diversity, although these analy-

ses have been limited to MSP-1 block 2. The first study was
carried out in 1990 in several regions of Colombia including
 Buenaventura, located in the western coastal region of Co-
lombia.22 In that study, most samples from Buenaventura
contained either the MAD20 or RO33 block 2 allelic types,
but K1 block 2 allelic type was not detected. A few samples
containing the K1 block 2 allelic type were detected in pa-
tients from other locations in Colombia. Nine of the 31
samples (29%) appeared to be mixed infections in that study,
while in our study 80% of the samples were mixed infections.
In a second study carried out in Chocó (northwest Colombia)
in 1997, all three MSP-1 block 2 allelic types were detected
although MAD20 was the predominant allele and K1 was the
least frequent.15 In studies carried out during 2000–2001 in
the Turbo and Zaragoza municipalities of northwestern and
northeastern Colombia, respectively, 100% of P. falciparum
samples contained only the MAD20 block 2 allelic type, simi-
lar to our studies in Buenaventura.23 The latter study and our
study, which were both carried out at approximately the same
time, suggest that current P. falciparum populations may have
become more homogeneous in several regions of Colombia.
However, our studies additionally detected a level of diversity
within MSP-1 block 4 in Colombian parasites that has not
been previously recognized.

Although we did not sequence block 4 in our study, se-
quence variation in block 4 was previously studied in parasite
clones from Thailand, the Brazilian Amazon, and several
common laboratory isolates.6,17 These results indicated that
recombination within block 4, although imprecise, was local-
ized to the central region of block 4 and appears to be the
only MSP-1 region that undergoes recombination resulting in
the generation of chimeric types. Although overall MSP-1
gene diversity in Buenaventura was lower than most other
hyperendemic and endemic areas studied in the same manner,
a higher average number of gene types per patient were ob-
erved in Buenaventura than reported previously for other
geographic areas. The detection of three of the four allelic
types found in Buenaventura in Vietnam, Brazil, and Tanza-
nia suggests that these MSP-1 gene types are broadly distrib-
uted worldwide. Frequencies of each of the four MSP-1 gene
types were significantly different for each geographic region,
suggesting that no single clone was of intrinsically greater
virulence than the others. It was previously suggested that
one of the mechanisms of selective pressure in malaria is the
host’s immunity against particular MSP-1 association types.24
Although it is possible that immunity developed after re-
peated or simultaneous, multiple infections decreases the
number of association types per patient with age, the stability
of multiple MSP-1 gene types within a population observed in
this and other studies suggest that MSP-1 block 4 is not under
strong positive or negative selection at the population level.
The persistence of both parental and recombinant alleles of
block 4 in the face of restricted heterogeneity over the rest of
the MSP-1 gene suggests that block 4 allelic diversity may
either be selectively neutral or under balancing selection.
Block 4 may represent a hot spot for meiotic recombination
that is neutral for selection either by the human immune sys-
tem or in the mosquito vector. Alternatively, balancing selec-
tion would favor maintenance of block 4 polymorphism for
longer periods of time as observed for the major histocom-
patibility complex genes of vertebrates.25 Both synonymous
d(S) and nonsynonymous (dNS) nucleotide substitution rates
across sites in different alleles of the MSP-1 gene indicate that
recombinants within sequences corresponding to region 4
such as seen in the CAMP isolate of P. falciparum have re-
sulted from relatively recent recombination events between
the K1 and MAD20 or closely related alleles.26 Balanced sele-
ction of Block 4 polymorphism may reflect the association
of these type-specific epitopes with different MHC class II
genes for CD4+ T cell recognition in a genetically diverse host
population.

Dominance of MAD20 sequences outside block 4, as indi-
cated by the prevalence of the MAD20 allelic type within
blocks 2, 6 and 16, suggests that there may be positive selec-
tion for the MAD20 allelic sequence outside of block 4 in P.
falciparum MSP-1. This is consistent with the higher preva-

ience of the MAD20 MSP-1 sequences encompassing blocks
6–17 in most malaria-endemic areas worldwide, including
southern Vietnam (82%), the Brazilian Amazon (96%), India
(94%), and all six populations examined in Africa (> 90%:
The Gambia, Nigeria, Gabon, northeastern Sudan, northeast-
er Tanzania, and South Africa).27,28 These studies have im-
lications for the design of a blood-stage malaria vaccine
based on MSP-1 because an effective vaccine should induce
an immune response specific for these dominant, MAD20-
related MSP-1 allelic sequences.
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