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Third Form of the Precursor to the Major Merozoite Surface Antigens of *Plasmodium falciparum*

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The precursor to the major merozoite surface antigens of *Plasmodium falciparum* appears to be encoded by two distinctly different (dimorphic) alleles able to undergo limited recombination. We analyzed 18 previously uncharacterized *P. falciparum* isolates to test the dimorphic model. All but one, a Thailand isolate, conformed to the dimorphic model, and this isolate conformed to the dimorphic model in all but variable block 2. Sequence analysis revealed that block 2 of isolate CSL2 was a third form. Hence, the dimorphic model is not strictly correct. Recombination between alleles was found only within two conserved blocks near the 5′ end of the gene.

The precursor to the major merozoite surface antigens (PMMSA; also known as the 195-kilodalton protein [3], the polymorphic schizont antigen [6], p190 [2], or the 200-kilodalton protein [7]) of *Plasmodium falciparum* varies antigenically between isolates (3, 6). DNA sequence analysis of the PMMSA gene from a number of isolates (4, 5, 7a, 9, 11) has revealed that the PMMSA sequence can be divided into blocks, some of which are conserved and others of which vary between isolates (7a, 9). The variable blocks studied so far occur in either of two distinctly different forms, so the antigen displays dimorphism (Fig. 1). Variable block 2 near the N terminus of the PMMSA contains a region of tandemly repeated amino acids which show a degree of polymorphism. In different isolates of *P. falciparum* a number of different combinations of these blocks exist as a result of meiotic recombination across the conserved blocks. Thus, the dimorphic nature of the PMMSA along with recombinational reassembling of the variable blocks and the polymorphic nature of the repeats can account for much of the antigenic variation in this molecule. As the PMMSA is an important candidate molecule for a malaria vaccine, we tested whether the dimorphic-allele model is true for a large number of isolates and determined the extent of recombination between dimorphic forms. We used oligonucleotide probes specific for each of the two forms of the seven variable blocks along the PMMSA to analyze the genotypes of 18 previously uncharacterized isolates of *P. falciparum*. Although the dimorphic model adequately describes 17 of these, a third distinct sequence occurs for block 2 of isolate CSL2. Hence, the diversity of this gene is greater than previously suspected.

Parasites. The origins of *P. falciparum* FC27, ItG2, 7G8, V1, NF7, K1, XP9, 1785, 1787, FCI1, MAD71, IMR143, IMR144, and IMR147 have all been described elsewhere (7a, 8). Isolates 3D7, HB3, and X5 were obtained from D. Walliker, Edinburgh, Scotland. Isolate PaloAlto was obtained from O. Mercereau Puijalon, Pasteur Institute, Paris, France. Isolates CSL2 and CSL3 were recently isolated from patients who had visited Thailand (CSL2) and Papua New Guinea (CSL3).

Preparation of DNA and oligonucleotide probes. Approximately 1 μg of DNA was prepared, denatured, and dotted onto nitrocellulose as described elsewhere (7a). Oligonucleotide probes (48-mers) were synthesized by annealing partially complementary 30-mers and were radiolabeled by filling in the overhanging ends as described elsewhere (7a). FC27-type variable-block probes were made corresponding to the following regions of the FC27 sequence (7a): block 2, nucleotides 280 to 327; block 4, nucleotides 1062 to 1109; block 6, nucleotides 1264 to 1311; block 8, nucleotides 2215 to 2262; block 10, nucleotides 2921 to 2968; block 14, nucleotides 3861 to 3908; and block 16, nucleotides 4768 to 4815. K1-type variable-block probes were made corresponding to the following regions of the K1 sequence (5): block 2, nucleotides 151 to 198; block 4, nucleotides 933 to 980; block 6, nucleotides 1120 to 1167; block 8, nucleotides 2059 to 2106; block 10, nucleotides 2681 to 2728; block 14, nucleotides 3582 to 3629; and block 16, nucleotides 4414 to 4461. Hybridization and washing conditions were as described elsewhere (7a). Autoradiography was performed at −70°C with two intensifying screens for 16 h.

Distinguishing alleles by use of dot blots. The variable blocks of the PMMSA differ to such an extent that oligonucleotides corresponding to one dimorphic form will not hybridize to the other and can therefore be used to type each of the variable blocks. The FC27 and K1 PMMSA DNA sequences, which differ at all seven variable blocks and thus can be considered the two parental types, were aligned, and regions of minimal homology within each variable block were selected. Oligonucleotides for each variable block were used to probe DNAs from 20 different isolates of *P. falciparum*. These probes proved to be very specific, as can be seen in the 11 examples shown in Fig. 2A to H. The nonreactive controls in Fig. 2A to H were *P. ovale* (spot 12), *P. berghei* (spot 13), and *P. vivax* (spot 14). The isolates of known structure (FC27, K1, and NF7) yielded the expected results.

Recombination within the PMMSA. For any pair of probes (FC27 type or K1 type) only one was observed to hybridize with a given isolate (e.g., Fig. 2A and B or Fig. 2C and D). In the examples shown in Fig. 2A to D, all of the isolates tested were either FC27-like (A and C) or K1-like (B and D) for both blocks 6 (A and B) and 16 (C and D), i.e., no recombinants were observed between these two blocks. Probing the same set of filters with oligonucleotides corresponding to blocks 2 and 14 identified a number of recombination events between these blocks (Fig. 2E to H). For example, the PaloAlto, X5, HB3, and ItG2 isolates were FC27-like in block 2 (Fig. 2E) and K1-like in block 14 (Fig.

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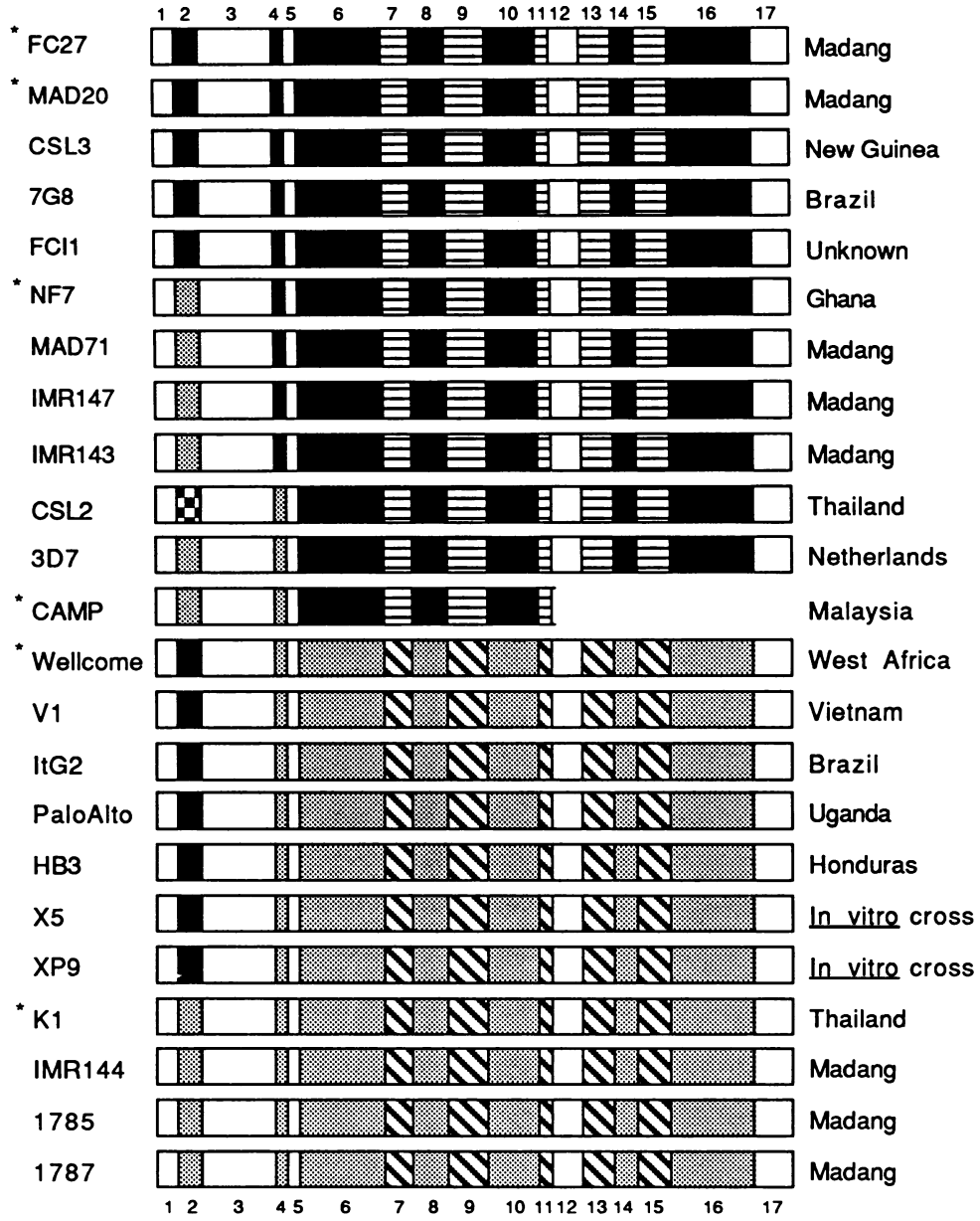

FIG. 1. Schematic of the amino acid sequences of 23 PMMSA molecules (the CAMP sequence is incomplete), using the convention of Tanabe et al. (9). The structures of the MAD20, CAMP, and Wellcome isolates were derived from their previously published sequences (9, 11, 14). All other variable boxes, represented either by filled boxes (FC27-like) or lightly shaded boxes (K1-like), were determined by using oligonucleotide probes. Conserved regions (open boxes) and semi conserved regions (boxes with horizontal and diagonal lines) were inferred from the dimorphic model (7a, 9). The sequences of isolates marked with an asterisk have been partially or completely determined.

2H), whereas the MAD71, IMR147, and IMR143 isolates were K1-like in block 2 (Fig. 2F) and FC27-like in block 14 (Fig. 2G).

With this analysis it was possible to unambiguously assign all seven variable blocks of all 20 isolates as either FC27-like or K1-like with one notable exception: CSL2 did not hybridize with either the FC27 or the K1 block-2 probe. The parents and two of the progeny of a genetic cross experiment (10) were included in this analysis. The parents, 3D7 and HB3, differed at all variable blocks except block 4 (Fig. 1), so a single recombination event within conserved block 3 or 5 could potentially be identified. Of the two progeny analyzed, X5 and XP9, both had the same genotype as HB3.

The results of the dot hybridizations are shown diagrammatically in Fig. 1 along with the structures deduced from the complete sequences of Wellcome, K1, and MAD20 and the partial sequences of NF7 and CAMP. (K1 and NF7 were also analyzed by dot blots.) There were clearly no recombination events between variable blocks 6 and 16 even though there was a conserved block (block 12) at which homologous pairing and recombination could potentially occur. Recombination events within blocks 3 and 5 were apparent (Fig. 1). If the FC27 and K1 sequences were considered to be the parental types, then a single recombination event within block 3 would result in progeny of the NF7 and Wellcome types. CAMP and 3D7 appeared to be progeny resulting

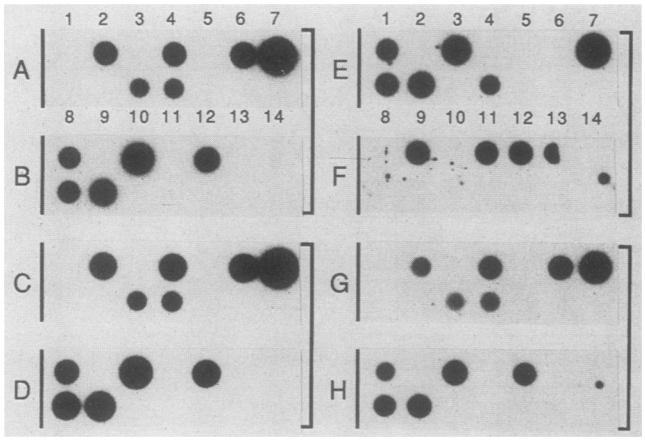

FIG. 2. DNA dot blot analysis of *Plasmodium* isolates. Procedures are described in Materials and Methods. Dots: 1, PaloAlto; 2, MAD71; 3, X5; 4, IMR147; 5, IMR144; 6, IMR143; 7, FC27; 8, ItG2 (1984); 9, ItG2 (1985); 10, CSL2; 11, CSL3; 12, *P. ovale*; 13, *P. bergei*; 14, *P. vivax*. Probes were as follows; A, FC27 block 2; B, K1 block 2; C, FC27 block 16; D, K1 block 16; E, FC27 block 2; F, K1 block 2: G, FC27 block 14; H, K1 block 14.

from a single recombination event between FC27 and K1 within block 5.

Sequence of a third form of the PMMSA. The failure of the block-2 probes to hybridize to isolate CSL2 was the single exception to the dimorphic-allele model for the PMMSA. To examine the molecular basis of this discordant result, we isolated and sequenced the *Eco*RI fragment containing the 5′ region of the PMMSA gene in CSL2 (Fig. 3). The CSL2 sequence has a structure similar to those of the FC27 and K1 sequences in this region, having conserved blocks 1 and 3 (5′ to the first asterisk and 3′ to the second asterisk in Fig. 3, respectively) and a block 2 which contains a stretch of repetitive DNA (arrows in Fig. 3). The CSL2 repeat is loosely based on a tripeptide repeat of Gly-X-Y or Ser-X-Y, where X is often Ala, but no two copies of the repeat in the CSL2 sequence are identical at either the DNA or the protein level. A comparison of the CSL2, FC27, and K1 sequences across the region of block 2 corresponding to the oligonucleotide probes used for this region (underlined in Fig. 3A) shows why neither of the probes hybridized to CSL2. The CSL2 sequence differs markedly from both the FC27 and K1 sequences in this region, sharing identity with the latter two sequences at only 27 of the 48 nucleotides contained in the block-2 probe.

Conclusions. We used oligonucleotide probes to test the dimorphic model of the PMMSA of *P. falciparum* proposed by Tanabe et al. (9) and supported by our recent observation (7a). Of the 23 isolates characterized by either DNA sequence analysis or hybridization with oligonucleotide probes, all except isolate CSL2 conform to the dimorphic-allele hypothesis. CSL2, isolated from a patient who had toured Thailand, has a region in block 2, the region of the PMMSA that includes the repetitive sequence, that does not hybridize to the probes. The probes do not correspond to the repeats but to the variable region just 5′ to the repeats. The DNA sequence revealed that the lack of hybridization to block 2 of CSL2 is due to a third type of block 2, not a deletion. Hence, the dimorphic model is not strictly correct: while it adequately describes all the other blocks, the existence of a third form of block 2 raises the possibility that many other forms may exist in nature.

A previous comparison of the DNA sequences of the PMMSAs from six isolates of *P. falciparum* indicated that recombination had occurred within conserved blocks 3 and 5 but not block 12 (7a, 9). Here we carried out a more extensive study of recombination within the PMMSA. No recombinants were observed between blocks 6 and 16 in 22 of the 23 isolates which are shown in Fig. 1 and for which sequence or hybridization data are available. This result suggests that such recombinants are selected against, presumably because they are nonfunctional. Of the 12 possible combinations resulting from single recombination events within blocks 3 and 5, 6 are observed among the 23 isolates shown in Fig. 1. These are represented by FC27, NF7, CSL2, 3D7, Wellcome, and K1.

We examined eight isolates arising from a localized geographical region, namely, the area surrounding Madang in the northern coast of Papua New Guinea. We detected three

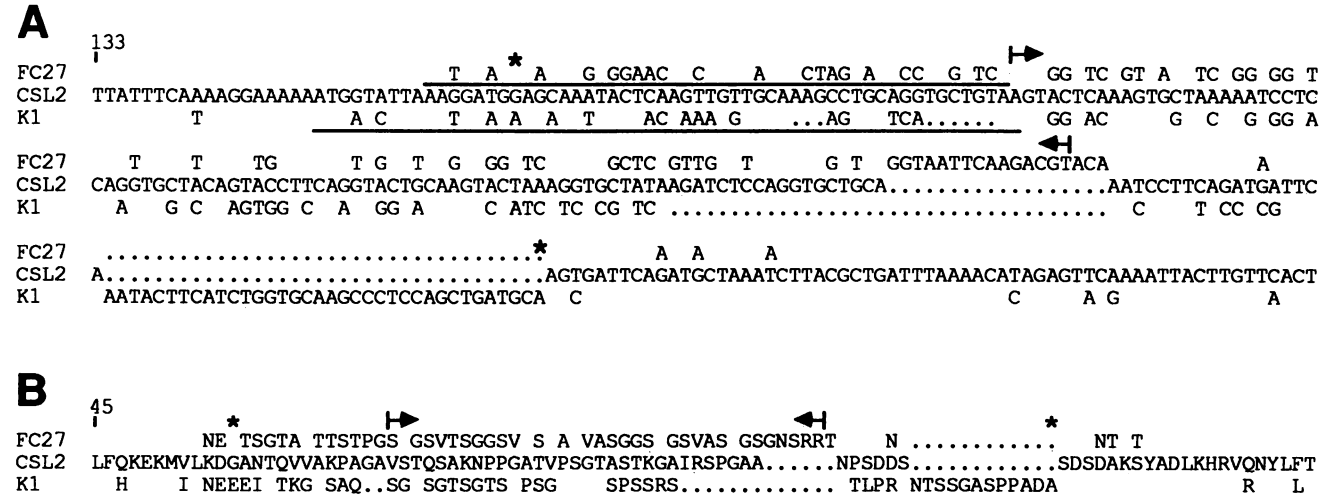

FIG. 3. DNA (A) and polypeptide (B) sequence comparisons of the homologous regions of the FC27, CSL2, and K1 PMMSA molecules. The DNA sequence alignment starts 133 base pairs downstream of the translational start, and the polypeptide sequence alignment starts at amino acid 45. The asterisks denote the boundaries of block 2, and the arrows denote the boundaries of the repeats (7a, 9).

distinct genotypes, as exemplified by FC27, MAD71, and IMR144. Thus, distinct genotypes of the PMMSA coexist in one small area. Conversely, the same genotypes of the PMMSA can be found in widely separated locations, e.g., Wellcome from West Africa, V1 from Vietnam, and ItG2 from Brazil are all of the same genotype. The hybridization approach used here provides an alternative that extends and complements the use of monoclonal antibodies (6). It is clear from both approaches that multiple forms can occur in a localized area and that individual forms can have a worldwide distribution. After submission of this manuscript, Certa et al. (1) reported a partial sequence of the PMMSA from a Ghanan isolate (RO-33), which contains a degenerate tripeptide repeat region almost identical to that of CSL2. However, RO-33 is not the same as CSL2, as it differs in having an FC27-type block 4 and thus extends the combinations of blocks found to 7.

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LITERATURE CITED

1. **Certa, V., D. Rotmann, H. Matile, and R. Reber-Liske.** 1987. A naturally occurring gene encoding the major surface antigen precursor p190 of *Plasmodium falciparum* lacks tripeptide repeats. EMBO J. **6**:4137–4142.
2. **Hall, R., J. McBride, G. Morgan, A. Tait, J. W. Zolg, D. Walliker, and J. Scaife.** 1983. Antigens of the erythrocytic stages of the human malaria parasite *Plasmodium falciparum* detected by monoclonal antibodies. Mol. Biochem. Parasitol. **7**:247–265.
3. **Holder, A. A., and R. R. Freeman.** 1982. Biosynthesis and processing of a *Plasmodium falciparum* schizont antigen recognized by immune serum and a monoclonal antibody. J. Exp. Med. **156**: 1528–1538.
4. **Holder, A. A., M. J. Lockyer, K. G. Odink, J. S. Sandhu, V. Riveros-Moreno, S. C. Nicholls, Y. Hillman, L. S. Davey, M. L. V. Tizard, R. T. Schwarz, and R. R. Freeman.** 1985. Primary structure of the precursor to the three major surface antigens of *Plasmodium falciparum* merozoites. Nature (London) **317**:270–273.
5. **Mackay, M., M. Goman, N. Bone, J. E. Hyde, J. Scaife, U. Certa, H. Stunnenberg, and H. Bujard.** 1985. Polymorphism of the precursor for the major surface antigens of *Plasmodium falciparum* merozoites: studies at the genetic level. EMBO J. **4**:3823–3829.
6. **McBride, J. S., C. I. Newbold, and R. Anand.** 1985. Polymorphism of a high molecular weight schizont antigen of the human malaria parasite *Plasmodium falciparum*. J. Exp. Med. **161**: 160–180.
7. **Perrin, L. H., B. Merkli, M. Locke, C. Chizzolini, K. J. Smart, and R. Richle.** 1984. Antimalarial immunity in saimiri monkeys. Immunization with surface components of asexual blood stage. J. Exp. Med. **160**:441–451.

7a. **Peterson, M. G., R. L. Coppel, P. McIntyre, C. J. Langford, G. Woodrow, G. V. Brown, R. F. Anders, and D. J. Kemp.** 1988. Variation in the precursor to the major merozoite surface antigens of *Plasmodium falciparum*. Mol. Biochem. Parasitol. **27**:291–302.

8. **Saint, R. B., R. L. Coppel, A. F. Cowman, G. V. Brown, P. T. Shi, N. Barzaga, D. J. Kemp, and R. F. Anders.** 1987. Changes in repeat number, sequence, and reading frame in S-antigen genes of *Plasmodium falciparum*. Mol. Cell. Biol. **7**:2968–2973.
9. **Tanabe, K., M. MacKay, M. Goman, and J. G. Scaife.** 1987. Allelic dimorphism in a surface antigen gene of the malaria parasite *Plasmodium falciparum*. J. Mol. Biol. **195**:273–287.
10. **Walliker, D., I. A. Quakyi, T. E. Wellems, T. F. McCutchan, A. Szarfman, W. T. London, L. M. Corcoran, T. R. Burkot, and R. Carter.** 1987. Genetic analysis of the human malarial parasite *Plasmodium falciparum*. Science **236**:1661–1666.
11. **Weber, J. L., W. M. Leininger, and J. A. Lyon.** 1986. Variation in the gene encoding a major merozoite surface antigen of the human malaria parasite *Plasmodium falciparum*. Nucleic Acids Res. **14**:3311–3323.