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Short communication

Minimal variation in the transmission-blocking vaccine candidate Pfs48/45 of the human malaria parasite *Plasmodium falciparum* ☆

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Recently we reported the isolation and characterization of the *Plasmodium falciparum* gene Pfs48/45 (strain NF54) that codes for a gametocyte/gamete-specific surface antigen, which is an important target antigen for transmission-blocking immunity [1–3]. To assess the suitability of this antigen for incorporation in a transmission-blocking subunit vaccine, it is important to know its antigenic diversity as it occurs in the field. Therefore we analyzed the nucleotide sequences of the Pfs48/45 genes in 7 in vitro cultivated *P. falciparum* strains collected from various geographic areas, i.e., Brazil (7G8) [4], Liberia (LE5, LF4) [5], Ghana (GH1), Honduras (HB3) [6], Malaysia (CAMP) [7], and Indochina (Dd2) [8], and compared these with that of NF54 [1], thought to be of African origin [9].

Immunological studies with Pfs48/45 specific monoclonal antibodies have shown that antigen Pfs48/45 contains at least 5 B-cell epitopes: I, IIa or IIc, IIb, III and IV [10–12], of which the first 4 are tertiary structure dependent. The only polymorphic epitopes identified thus far are IIa and IIc, which behave as though they are mutually exclusive [12]. Because epitope IIa is absent from gene Pfs48/45 of strain NF54, but present in that of 7G8 [11,12], and conversely epitope IIc is absent from 7G8 but present in NF54 [12] (Pieter Beckers and Ton Lensen, personal communication), the entire nucleotide sequence of the coding region of the intronless gene Pfs48/45 of 7G8 was established. First, the coding region (1347 base pairs) was amplified with the polymerase chain reaction (PCR) using primers previously described [1]. Subsequently the PCR products were sequenced without intermediate subcloning

Abbreviations: PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism; *Taq*, *Thermus aquaticus*

Note: Nucleotide sequence data reported in this paper are available in the EMBL, GenBank™ and DDJB data bases under the accession numbers Z22145 and X81648.

☆ This publication is dedicated to John G.G. Schoenmakers, who passed away on July 13, 1994.

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A

			Epitope
NF54	751 251	TAT CAT AAA AAC TTA ACT ATC 771 Tyr His Lys Asn Leu Thr Ile 257	IIa⁻/IIc⁺
7G8, CAMP		... ... G.. ..G ... ... Glu Lys	IIa⁺/IIc⁻
Dd2, HB3		... ...G ... ... Lys	IIa⁺/IIc⁻
GH1, LF4, LE5		... ...	IIa⁻/IIc⁺

B

NF54	904 302	TCA AAT GTT AGT TCT AAA CAT ACT TTT ACA GAT AGT TTA GAT Ser Asn Val Ser Ser Lys His Thr Phe Thr Asp Ser Leu Asp
Dd2		... ... .A. ... ... Asp
LF4, LE5		... ... A.. ... Ile
HB3		... ...
NF54	946 316	ATT TCT TTA GTT GAT GAT AGT GCA CAT ATT TCA TGT AAC GTA Ile Ser Leu Val Asp Asp Ser Ala His Ile Ser Cys Asn Val
Dd2, LF4, LE5		... ...
HB3		... ... .A. ... ... Asn

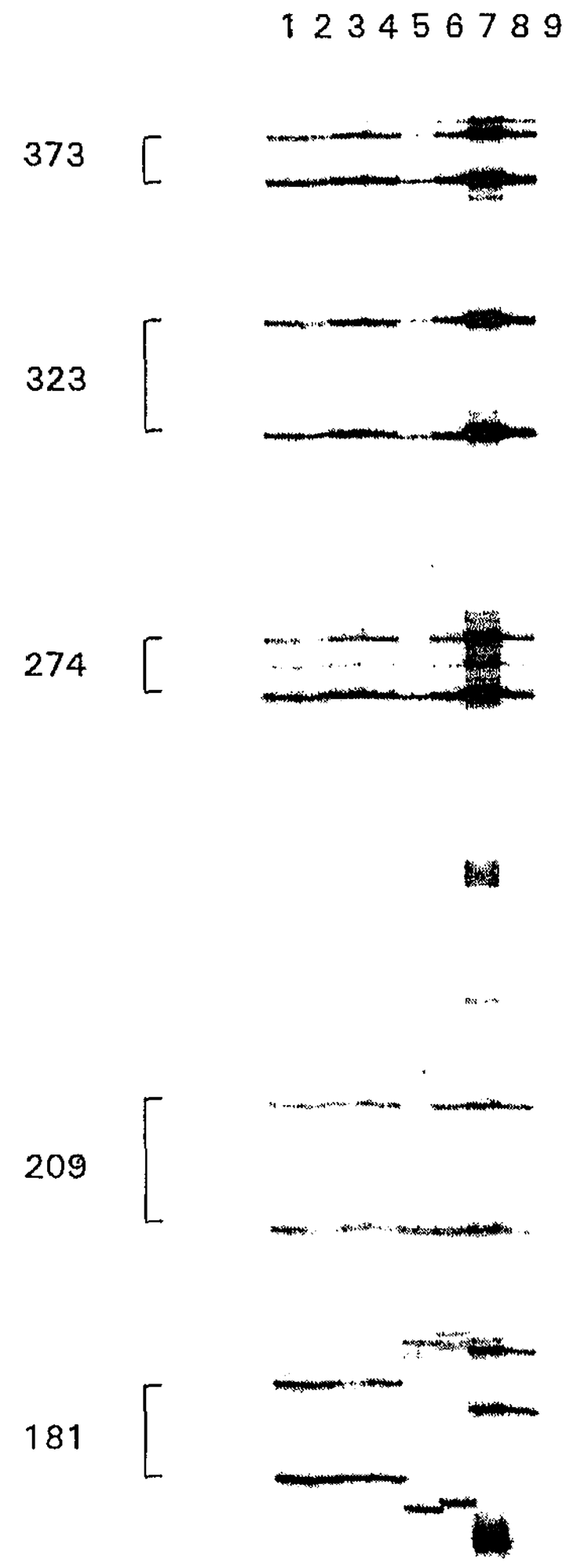
Fig. 1. Nucleotide and amino acid sequence differences between the Pfs48/45 genes of 8 different strains of *P. falciparum*. (A) Comparison of the nucleotide and amino acid sequence of the putative epitope region IIc of NF54 with those of the other 7 strains examined. Only nucleotides and deduced amino acids that are different, are indicated. Whether (part of) the amino acid sequence of this region is recognized by monoclonal antibodies that are specific for epitope region IIa or IIc, is also indicated [10–12]. (B) Nucleotide and amino acid sequence of the polymorphic region in Pfs48/45 of NF54 as detected by SSCP analysis and comparison with the nucleotide and amino acid sequences in the respective strains examined. Only nucleotides and amino acids that are different are indicated.

[13]. Between the Pfs48/45 genes of 7G8 and NF54 only 2 nucleotides were found to be different (Fig. 1A). In 7G8 at nucleotide position 757 there is a G instead of an A, and at nucleotide position 762 a G instead of a C, with the consequence that the consecutive amino acids lysine and asparagine at positions 253 and 254 in Pfs48/45 of NF54 are replaced by a glutamic acid and lysine residue, respectively, in 7G8.

Because epitopes other than IIa and IIc are highly conserved [10–12], initially of the other strains only the DNA region of Pfs48/45 that is different in NF54 and 7G8 (see above) was sequenced (Fig. 1A). In CAMP the sequence of this region is identical to that of 7G8, while in GH1, LF4 and LE5, of which the latter two have the same epitope structure as NF54 [10,11], the sequence of this region is identical to that of NF54. The Pfs48/45 genes of the strains that possess the same epitope structure as 7G8 [12], i.e., Dd2 and HB3, when compared to Pfs48/45 of NF54 contain at position 762 a G instead of a C, and consequently at amino acid position 254 a lysine instead of an asparagine residue.

Taken together, the results of the immunological [10–12] and structural studies (Fig. 1A) suggest that the amino acid residue at position 254 in Pfs48/45 dictates whether antigen Pfs48/45 will bind to a monoclonal antibody that is specific for epitope IIa or epitope IIc.

Whether these differences in affinity are caused by differences in the conformations of the respective epitopes, or due to a differential posttranslational modification, awaits further investigation.



To investigate whether the Pfs48/45 genes of the respective strains differ at other nucleotide positions, polymorphic regions were identified using the single-strand conformation polymorphism (SSCP) technique on PCR-products [14,15]. Because gene Pfs48/45 (1344 bp) is too long for reliable SSCP-analyses, several restriction enzymes were used to cleave the PCR-amplified gene into subfragments that are smaller than 400 bp [14]. Only one single polymorphic region was observed, which surprisingly (see below) was found to be situated within the 181-bp-long *Fok*I fragment (nucleotide positions 903–1084) of a *Fok*I-*Sau*3a digest (Fig. 2), and after comparison of all SSCP-data (see legend to Fig. 2), also within a 83-bp-long *Fok*I-*Rsa*I fragment (nucleotide positions 903–986). For unknown reasons (see also [15], in which similar observations are described) polymorphic fragments that contained the nucleotide changes identified in Fig. 1A were not detected by this technique. Thus if one accepts the assumption that the latter region is the only polymorphic region that escaped detection in the SSCP-analyses, it can be concluded that, with the exception of the nucleotide differences already given in Fig. 1A, the nucleotide sequence of Pfs48/45 in GH1, 7G8 and CAMP is identical to that of NF54. Upon sequence analysis of the 83-bp *Fok*I-*Rsa*I fragment of Dd2, HB3, LF4 and LE5, only single nucleotide differences were noted from NF54 (Fig. 1B). LF4 and LE5 have the same T-A transversion at nucleotide position 940, resulting in a conservative amino acid substitution. Dd2 has a T-A transversion at position 911 and HB3 a G-A transition at position 965, resulting in both cases in a non-conservative amino acid substitution. The data presented, together with the results of the immunological studies published previously [10–12], show clearly that, similarly to the sexual stage specific surface antigens Pfs25 [16] and Pfs230 [17], the antigenic diversity of Pfs48/45 as it occurs in the field is exceedingly low. This observation further strengthens earlier conclusions that Pfs48/45 is an important candidate antigen for the development of a transmission-blocking subunit vaccine.

Fig. 2. SSCP-analysis of DNA fragments obtained after incubation of PCR-amplified Pfs48/45 DNA with the restriction endonucleases *Fok*I and *Sau*3A. Lanes: single-stranded DNA fragments of (1) NF54, (2) GH1, (3) CAMP, (4) 7G8, (5) Dd2, (6) HB3, (7) LF4, (8) LE5. Products of a negative control experiment were analyzed in lane 9. Sizes of the fragments in nucleotides (nt) are indicated. Note that the 181 nt long (*Fok*I) fragment is the only polymorphic fragment detected. The Pfs48/45 coding region was PCR-amplified [1] and subsequently fragmented with restriction enzymes. After dissolution of the fragments in 3 μl H_2O and 15 μl of 95% formamide/0.05% bromophenol blue/0.05% xylene cyanol, they were heat denatured (5 min at 100°C) and after chilling in ice-water loaded (4 μl) onto a 6% nondenaturing polyacrylamide gel containing 45 mM Tris-borate, pH 8.3/4 mM EDTA/5% glycerol [14]. After electrophoresis (Pharmacia/LKB Macrophor; 45 W at constant power, 6–7 h at 18°C), the gel was dried and subjected to autoradiography. To assure that none of the polymorphic regions would escape detection, a total of six different digests (*Fok*I/*Sau*3A, *Alu*I/*Mbo*II, *Ssp*I, *Rsa*I, *Acc*I/*Dra*I/*Sau*3A and *Alu*I/*Acc*I/*Ssp*I) were analyzed, some both at 22°C and 18°C (data not shown). For some fragments different conformers of the same sequence were found at 18°C [14], resulting in more than two bands per restriction fragment (compare for example the bands for the fragments that are, respectively, 209 nt and 274 nt long). The finding of faint bands proximal to the 209 nt fragments in GH1 and 7G8 (lanes 2 and 4, respectively) was not reproducible. 'Ghost' bands were also observed in genomic DNA digests of other strains and no sequences different from NF54 was found in this fragment of 7G8. The same is true for the faint bands around the 181 nt long fragment of NF54 and CAMP (lanes 1 and 3, respectively).

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