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IMMUNOGENICITY AND ANTIGENICITY OF THE N-TERM REPEAT AMINO ACID SEQUENCE OF THE *PLASMODIUM FALCIPARUM* P126 ANTIGEN

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The P126 protein, a parasitophorus vacuole antigen of Plasmodium falciparum *has been shown to induce protective immunity in* Saimiri *and* Aotus *monkeys. In the present work we investigated its immunogenicity. Our results suggest that the N-term of P126 is poorly immunogenic and the antibody response against the P126 could be under a MHC restricted control in C57BL/6(H—2b) mice, which could be problematic in terms of a use of the P126 in a vaccine program. However, we observed that a synthetic peptide, copying the 6 octapeptide repeat corresponding to the N-term of the P126, induces an antibody response to the native molecule in C57BL/6 non-responder mice. Moreover, the vaccine-P126 recombinant induced antibodies against the N-term of the molecule in rabbits while the unprocessed P126 did not.*

Key word: *Plasmodium falciparum* – P126 – synthetic peptide – vaccine-P126 recombinant – immunogenicity – immunization

The P126 protein of *Plasmodium falciparum* (Delplace et al., 1985) has been described independently by various authors as either a 140 kDa protein (Perrin et al., 1984), a 113 kDa protein (Chulay et al., 1987), the SERP (Knapp et al., 1989) or the SERA (Bzik et al., 1988).

The P126 is synthesized by the parasite between the 32nd the 36th hour of a 42 hour erythrocytic cycle and is stored inside the parasitophorous vacuole (Delplace et al., 1987). The P126 is processed when schizont-infected erythrocytes rupture to give on one hand a 73 kDa fragment that is composed of two peptides of 47 and 18 kDa linked by disulfide bridges and on the other hand a 56 kDa fragment that is rapidly processed in a 50 kDa fragment (Fig.). The processing phenomenon that is responsible of the 56-50 fragment transformation is associated with the release of merozoites (Debrabant & Delplace 1989).

The P126 protein is characterized by a highly conserved amino acid sequence among different strains, and repetitive sequences, consisting of (i) a poly-serine region and (ii) six octapeptides at the N-term of the molecule i.e. at the N-term of the 47 kDa sub-unit of the P73 (Weber et al., 1987; Bzik et al., 1988; Coppel et al., 1988).

Perrin et al. (1984) described first the induction of a protective immunity against a malarial challenge infection in *Saimiri* monkeys using the protein purified by electro-elution. Delplace et al. (1988) reported a partial immunization of *Saimiri* monkeys using the P126 purified by immuno-affinity and coupled to alumine hydroxyde. More recently, Inselburg et al. (1991) were able to protect Aotus monkeys using recombinant proteins corresponding to the N-term of the molecule. Therefore, the P126 antigen is considered as a potential component of a malaria vaccine. However, the characterization of the effector mechanisms responsible of the induced immunity has not yet been clearly established (Delplace et al., 1988). In particular, it is not known whether the immunity is supported by an inhibition of the 56-50 fragment transformation. On the contrary, a monoclonal antibody specific of the N-term of the molecule, *i.e.* corresponding to the 47

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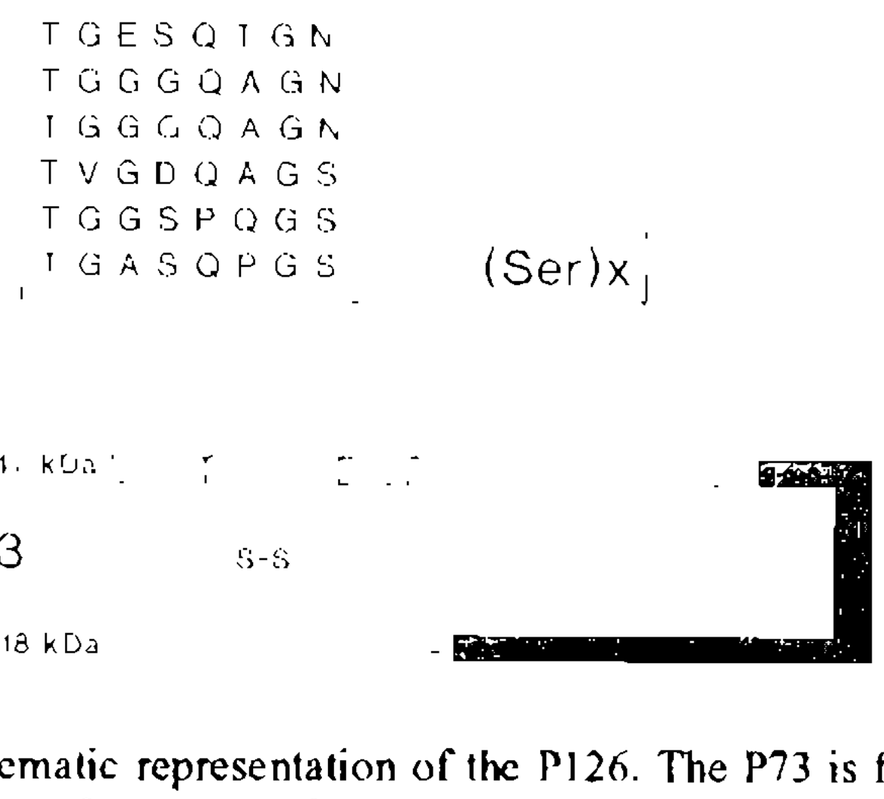

Schematic representation of the P126. The P73 is formed of two fragments of 47 and 18 kDa linked by disulfide bonds. The 47 kDa contains the 6 octapeptide repeat at the N-term and the polyserinbe region.

kDa fragment, has been shown to inhibit the growth of the parasite *in vitro* (Horii et al., 1988).

In the present work we investigated the immunogenicity of: (1) the unprocessed P126, contained in unruptured schizonts and purified either by a 2-step immuno-affinity or electro-elution after a first immuno-affinity; (2) the P73 and P50 fragments, which are released in the culture supernates and which can be purified by electro-elution; and (3) the N-term repeated sequence of the molecule, using a synthetic peptide copying the 6 octapeptide repeat (called Nt-47).

It has not been possible to test the polyserine region since the corresponding synthetic peptide is insoluble in physiological solutions.

We first observed that Balb/c mice immunized with (i) schizonts or culture supernates produced antibodies against the P126, (ii) the P73 or the P50 produced antibodies against the corresponding fragments and the P126, and (iii) the Nt-47 synthetic peptide produced antibodies against the P47 and the P126. However, no antibodies against the Nt-47 were detected in sera from mice immunized with purified schizonts while mice immunized with culture supernates generated antibodies against the N-term of the 47 kDa fragment (Table). Taken together, these results suggest that the N-term of the molecule, inside the unprocessed P126 is not, or poorly, immunogenic.

Following these experiments, Balb/c, CBA and C57BL/6 mice were immunized with purified schizonts or culture supernates. We observed that C57BL/6 mice produced antibodies against various antigens contained in these preparations but not against the P126, while Balb/c and CBA mice generated antibodies against the P126. The specific unresponsiveness against the P126, observed in C57BL/6 mice, suggests that the antibody response against the molecule could be under a MHC restricted control as observed for various *Plasmodium* antigens (recently reviewed by Riley et al., 1991). This observation should lead to further investigations in *P. falciparum* infected humans in order to determine whether an MHC restricted control could occur for the P126 protein. In fact, 80% of sera from infected humans contain antibodies against the P126 and 70% out of these sera are able to recognize the Nt-47 synthetic peptide without difference between sera from prime infected or chronically exposed individuals.

Additional experiments have shown, on the contrary, that specific P126 antibodies can be produced in C57Bl/6 mice using either electro-eluted P50 or the Nt-47 synthetic peptide for immunization.

Another interesting observation has also been obtained with rabbits. A rabbit, which has been immunized with P126 purified by immuno-absorption, was able to produce antibodies against the P126 but not against the N-term of the molecule as observed using a Nt-47 ELISA. However, (i) a rabbit immunized with the Nt-47 produced antibodies against the peptide and the native molecule, (ii) a rabbit immunized with a vaccine-P126 recombinant produced antibodies against the P126 molecule and its fragments as well as the N term of the molecule (see the paper from C. de Taisne).

Our results suggest that the N-term of the P126 is poorly immunogenic and the antibody response against the P126 is under a MHC restricted control, which could be problematic in terms of a use of the P126 in a vaccine program. However, it is highly encouraging to note that constructs like the Nt-47 synthetic peptide or the vaccine-P126 recombinant are more immunogenic than the native molecule: the Nt-47 induces an antibody response in C57B1/6 non-responder to the P126 native molecule and the vaccine-P126 recombinant induces antibodies against the N-term of the molecule in rabbits while the immunopurified P126 does not.

TABLE

Detection of antibodies against *Plasmodium falciparum* P126, its fragments or unrelated antigens using western-blot (WB) of schizonts (schzt) or culture supernatant (supt), immunoprecipitation (Ippt) or ELISA with the synthetic peptide Nt-47, in sera of various mice immunized with purified schizonts, culture supernantant, immunopurified P126 or immunopurified P50 by electro-elution

Immunization with	Assay	Antibodies detected in Balb/c ($H2^d$) CBA($H2^k$)	Antibodies detected in C57BL/6 ($H2^b$)
Purified schizonts	W.B. (schzt)	P126 +	P126 –
		Other Ags +	Other Ags +
	W.B. (sup^t)	P73 +W, P50 +	P73 –, P50 –
		Other Ags +	Other Ags +
	Ipp^t (schzt)	P126 +	P126 –
		Other Ags +	Other Ags +
	ELISA (Nt–47)	Negative	Negative
Culture supernatant	W.B. (schzt)	P126 +	P126 –
		Other Ags +	Other Ags +
	W.B. (sup^t)	P73 +, P50 +	P73 –, P50 –
		Other Ags +	Other Ags +
	Ipp^t (schzt)	P126 +	P126 –
		Other Ags +	Other Ags +
	ELISA (Nt–47)	Positive	Negative
P126 Immunopurified	W.B. (schzt)	P126 +	P126 +
	ELISA (Nt–47)	Negative	Negative
P50 Electro-eluted	W.B. (schzt)	P126 +	P126 +
	W.B. (sup^t)	P50 +	P 50 +
	Ipp^t (schzt)	P126 +	P126 +
	ELISA (Nt-47)	Negative	Negative

Balb/c, CBA and C57B1/6 mice were immunized with either purified schizonts, culture supernatant, immunopurified P126 or electro-eluted P50. Antibodies were detected on Western blot of schizonts (W.B. schzt), on Western blot of culture supernatants (W.B. sup^t), by immunoprecipitation of radio-labeled schizonts (Ipp^t schzt) or by ELISA using the Nt-47 synthetic peptide (EISA)| Nt-47). Positive and negative reactions are respectively reported + or -. Weak reaction are noted +W. Reactions against antigens different of the P126 are noted "Other Ags".

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