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Passive transfer of *Plasmodium falciparum* MSP-2 pseudopeptide-induced antibodies efficiently controlled parasitemia in *Plasmodium berghei*-infected mice

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ABSTRACT

We have developed monoclonal antibodies directed against the pseudopeptide ψ-130, derived from the highly conserved malarial antigen *Plasmodium falciparum* merozoite surface protein 2 (MSP-2), for obtaining novel molecular tools with potential applications in the control of malaria. Following isotype switching, these antibodies were tested for their ability to suppress blood-stage parasitemia through passive immunization in malaria-infected mice. Some proved totally effective in suppressing a lethal blood-stage challenge infection and others reduced malarial parasitemia. Protection against *P. berghei* malaria following Ig passive immunization can be associated with specific immunoglobulins induced by a site-directed designed MSP-2 reduced amide pseudopeptide.

1. Introduction

Merozoite surface proteins (MSP) have been considered as potential vaccine candidates against *Plasmodium* spp. blood-stages due to their easy accessibility which turns them into excellent immune system's targets [40]. Among these surface proteins interacting with erythrocyte membrane during invasion, the MSP-2 has gained notable attention as a source of vaccine candidate peptides, since the reactivity of antibodies acquired by humans during natural infection against this protein is associated with clinical immunity against malaria [40]. The MSP-2 is a 35–56 kDa glycoprotein whose size and isoelectric point vary depending on the parasite strain. It is displayed over the surface of merozoites and in residual bodies from all *Plasmodium falciparum* strains studied so far [6,45]. It possess a highly polymorphic central region flanked by dimorphic sequences of either ICI or FC27 allelic families, which are in turn flanked by conserved sequences [13,46,50] as observed in Fig. 1A.

The isotype of antibodies produced in response to *P. falciparum* MSP-2 during infection generally reflects the type of parasite allele causing the infection and the length of exposure [36,49,52]. Some studies have shown that monoclonal antibodies directed against MSP-1 and MSP-2 surface antigens inhibit *in vitro* parasite growth [10,25,39], while others have established that immunizing mice with peptides from the *P. falciparum* MSP-2 N-terminal region makes them capable of protecting themselves against *P. berghei* and *P. yoelii* strains [24,43]. It has been reported that a MSP-2 N-terminal region peptide (namely 4044) bound with high specificity to human erythrocytes and possesses three binding motifs [34]. Bearing in mind that humoral immunity plays an important role in the defense against blood-stage infections [3–5,11,14,27,30,37,41,44], passive immunization is considered to be a potential strategy in the search for epitopes and in studying the mechanisms by which antibodies provide a protective effect against a pathogen-causing disease. Steric interference in merozoite invasion of erythrocytes, inhibition of intra-erythrocyte development or processing of merozoite surface proteins, antibody-depend cell inhibition and phagocytosis are among the mechanisms proposed to date for explaining how antibodies achieve their effect [9,28,39,54,55]. Studies on mice have shown that passive immunization with antibodies directed against *P. yoelii* AMA-1, CS, MSP-1, proteins confer protection [31,32,47]. It has been demonstrated *in vitro* that IgG antibodies from African adults immune to *P. falciparum* have inhibited parasite growth in cooperation with monocytes; which was correlated with reduced *in vivo* parasitemia [3].

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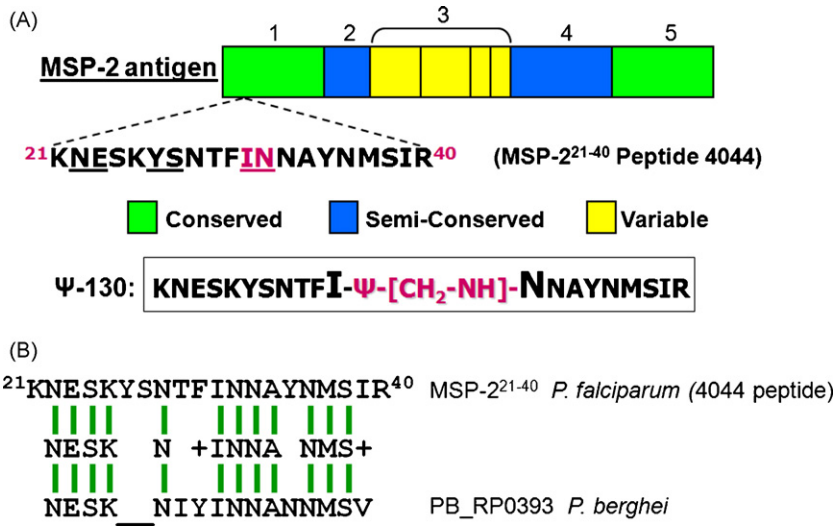

Fig. 1. Structural characteristics of the MSP-2-derived peptide 4044. Schematic representation of the *Plasmodium falciparum* merozoite surface protein-2 in which the peptide 4044 primary structure is shown, as well as its genetic organization containing conserved domains (blocks 1 and 5), non-repetitive dimorphic domains (blocks 2 and 4), and the central polymorphic repeats (block 3) (A). Amino acid alignment between peptide 4044 and the whole genome shotgun sequence of *Plasmodium berghei* ANKA strain as reported in the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/) (B).

Since eliciting protection-inducing antibodies depends upon the antigen's nature, previous work has established that immunizations with *P. falciparum* MSP-1 reduced amide pseudopeptides led to producing monoclonal antibodies able of inhibiting *P. falciparum in vitro* invasion of and growth into human red blood cells (RBCs) compared to specific antibodies against the native peptide [25]. These malaria pseudopeptides' immuno-modulating properties result from replacing a naturally produced peptide-bond by a reduced amide form, thus inducing new structure features in the target molecule as a result of replacing the normal trigonal sp2 planar geometry of an amide bond by four new sp3 hybridized bonds which modifies the entire molecule's 3D structure. A desirable side-effect of such a geometrical change is the new molecule's increased stability to sera endo-proteases. Modifying the peptide bond thus produces a less biodegradable peptide bond, influencing molecules' immuno-biological properties without modifying the amino acids' side-chains so important for appropriate molecular recognition and thus transforming these molecules into potential transition-state analogues as efficient protease blockers [25].

Bearing in mind that proteolytic processing of specific parasite surface antigens is essential during infection, then blocking such processing could be a strategy leading to evading certain infections [22].

This work was thus aimed at producing specific site-directed antibodies against the ψ-130 isoster-bond pseudopeptide derived from the native sequence of the High Activity Binding Peptide (HABP) coded 4044 peptide and the subsequent assessment of these antibodies protection-inducing ability by passive immunization experiments conducted in mice. Further use of passive immunization with site-directed and specifically designed antibodies could be a useful immuno-therapeutic strategy in areas where malarial strains are resistant to antibiotics and other pharmacological agents for achieving control of this deadly disease.

2. Materials and methods

2.1. Bioinformatics analysis

Both the *P. falciparum* MSP-2 protein sequence (gi: 1222687) as well as the peptide 4044 *P. falciparum* MSP-2-derived sequence were aligned against the whole genome shotgun sequence of *Plasmodium berghei* ANKA strain reported in the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/), by using the TBLASTN tool.

2.2. Isoster peptide bond-immunogen synthesis

Based on the MSP-$2^{21–40}$ (peptide 4044) primary structure observed in Fig. 1, a set of reduced amide pseudopeptides was designed and synthesized as described bellow. The non-modified as well as its pseudopeptide surrogates were obtained in both monomer and polymer forms. All molecules were manually synthesized using a Merrifield's previously reported protocol for t-Boc-based solid-phase peptide synthesis (SPPS) [28], this procedure was later modified for multiple-peptide synthesis [17]. Each amino acid residue was placed on the pseudopeptide backbone as has been described elsewhere [7,16,42]. The ψ-[CH_2NH] surrogate was introduced by the deprotected N^{α}-amino group's resin-bound reductive alkylation with the t-Boc-protected amino acid aldehyde (0.576 mmol) in DMF containing 0.5% HOAc followed by portion-wise addition of $NaBH_3CN$ (0.67 mmol) for 40–60 min. The resin was checked for a totally allowed coupling by the Ninhydrin test and, when necessary, the coupling reaction was repeated until completeness. Coupling was allowed to proceed for 5 h with constant stirring, followed by *N*,*N*′-dimethylformamide (DMF), isopropanol and dichloromethane washing. Standard solid phase peptide synthesis was carried out to introduce the remaining t-Boc amino-acids to the N-terminal final residue. Protected pseudopeptide-resin batches were treated with TFA and cleaved from the resin by treatment with low concentrations of anhydrous HF containing 10% anisole at 0 °C for 60 min. After HF evaporation in an N_2 stream, each pseudopeptide-resin product was washed with cold diethyl ether, then extracted with 5% HOAc and lyophilized. The crude products obtained for each ψ-[CH_2NH] surrogate were further analyzed by analytical RP-HPLC, purified by preparative RP-HPLC and identified by MALDI-TOF mass spectrometry. A full procedure for peptide-based polymer production is elsewhere described [23,25].

2.3. Mice immunization for poly- and monoclonal antibody production

The pseudopeptide ψ-130 having a methylene amine isoster bond between residues ^{31}I-N^{32} from preliminary tests was further