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Phi (Φ) and psi (Ψ) angles involved in malarial peptide bonds determine sterile protective immunity

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ABSTRACT

Modified HABP (mHABP) regions interacting with HLA-DRβ1* molecules have a more restricted conformation and/or sequence than other mHABPs which do not fit perfectly into their peptide binding regions (PBR) and do not induce an acceptable immune response due to the critical role of their Φ and Ψ torsion angles. These angle's critical role was determined in such highly immunogenic, protection-inducing response against experimental malaria using the conformers (mHABPs) obtained by ^{1}H-NMR and superimposed into HLA-DRβ1*-like *Aotus* monkey molecules; their phi (Φ) and psi (Ψ) angles were measured and the H-bond formation between these molecules was evaluated. The aforementioned mHABP propensity to assume a regular conformation similar to a left-handed polyproline type II helix ($PPII_L$) led to suggesting that favouring these conformations according to their amino acid sequence would lead to high antibody titre production and sterile protective immunity induction against malaria, thereby adding new principles or rules for vaccine development, malaria being one of them.

1. Introduction

Unfortunately, a long-expected and sought-after vaccine against *Plasmodium falciparum* malaria parasite blood stages has yielded disappointing results to date [1–4], thereby highlighting the molecular and biological complexity of this parasite that causes around 200 million cases and 1.2 million death a year [5]. Almost all vaccine candidates consist of biologically-derived products using recombinant proteins, DNA, vector-based fragments inserted in viruses, bacteria or minimal parasite doses [3,4].

SPf66 was the first multi-epitope, multistage, minimal subunit-based, chemically-synthesised antimalarial vaccine (developed 25 years ago) that provided sterile protective immunity in the *Aotus* experimental model [6], human challenges [7] reaching phase III large-scale field-trials in different parts of the world [8–10]. Since then we have insisted that the most reliable and expeditious approach to a logical and rational methodology for vaccine development is a complete and deep understanding of the *P. falciparum* malaria parasite and the human host's immunological system (at subatomic level if possible) due to their tremendous complexity. This manuscript was thus aimed at furthering new principles and updating rules for this purpose.

Our institute's 32 year's experience in this field has thoroughly shown that identifying aminoacid sequences derived from the most relevant proteins involved in merozoite binding to and invasion of red blood cell (RBC) is the first step in the recognition of epitopes to be included as minimal subunit components in a fully-protective vaccine [11]; such aminoacid sequences have been named **h**igh **a**ctivity **b**inding **p**eptides (HABPs). We have also shown that conserved HABP (cHABP) critical binding residues (CBR) have to be replaced by others having similar mass and volume but opposite polarity to induce a protective immune response (CBR binding capacity drops by >50% when replaced in a glycine analogue scanning test) [12]. CBR establishing H-bonds with other cHABPs in the same protein [11,13] or with receptor molecules on target cells to be infected by this route must also be replaced [14].

These modifications have led to a better fit into major histocompatibility complex class II (MHCII) molecules, also named HLA-DR, DQ, DP in humans or *Ao*-HLA-like in *Aotus* monkeys (displaying 90–100% similarity with their human counterparts [15]) forming the peptide–MHC complex (pMHC) to be presented to T-cell receptor (TCR) molecules and thus establish an appropriate MHCII–p–TCR complex to properly induce an effective immune response [16].

Extremely well thought-out, elegant work (at atom level) by several groups using X-ray crystallography to determine class II or HLA-DR molecule 3D structure [17–20], the MHC–p–TCR complex [21–23] and the conserved residues interacting amongst them [24,25] has led to better understanding of physical and chemical rules for antigen presentation and the corresponding immune responses.

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Our ongoing search for such logical and rational methodology during the last 15 years has resulted in high antibody titres and sterile protective immunity being elicited in the *Aotus* monkey (an appropriate experimental model as it is highly susceptible to human malaria); and the 3D structure of ~150 cHABPs and their corresponding modified HABPs (mHABPs) being determined by ^{1}H NMR and this, in turn, has led to fundamental principles and rules being established for effective vaccine development [26].

Our group has very recently identified mHABP stereo-electron effect differences in mHABPs [27]; this manuscript thus describes further physical–chemical characteristics for these highly immunogenic and protection-inducing mHABPs in which their peptide bond phi (Φ) and psi (Ψ) dihedral angles have been seen to be very relevant in terms of their 3D structure. This allows an appropriate fit into HLA-DR molecules, thereby eliciting high antibody titres and sterile protective immunity against malaria, thus providing new and complementary physical–chemical rules for vaccine development at subatomic level.

2. Materials and methods

Monomer and polymer cHABPs (in parenthesis, not in bold) or mHABPs (shown in bold) produced by following Merrifield's classical peptide synthesis methodology [28] and numbered according to our institute's serial numbering system have been thoroughly described [26]. Immunological studies, such as immunofluorescent antibody (Ab) test (IFA) and Western blot (WB) analysis, have been performed with sera from *Aotus* monkeys immunised with polymer HABPs [26,29–31]. The monkeys were kept in FIDIC's field-station in the Colombian Amazon Jungle (legal permits having been granted by the Colombian government environmental authorities INDERENA and CORPOAMAZONIA since 1984, the last Nr 0266 being issued in December 2010) and maintained according to National Institute of Health guidelines for animal handling. They were constantly supervised by CORPOAMAZONIA officials when in captivity and returned to the jungle after the experiments finished, in excellent conditions of health.

The ^{1}H NMR structure of mHABP **a**pical **m**erozoite **a**ntigen-1 (AMA-1) **10022** (4313) [31], merozoite surface protein 2 (MSP-2) **24112** (4044) [29] and merozoite surface protein 1 (MSP-1) **10014** (1585) and their ability to bind to purified HLA-DR molecules have been described in the aforementioned references; they have all been derived from very relevant proteins involved in RBC invasion [30].

A group of conformers obtained by ^{1}H NMR was analysed using INSIGHTII (ACCELLRYS Inc, software, USA); those having the lowest energy, displaying the HLA-DRβ1binding motifs and binding registers were chosen [32]. They have been indicated by our serial number followed by a dot (Fig. 2). Their Φ and Ψ angles as measured in the ^{1}H NMR spectra were exported in format .wrl, to be rendered in 3D studio MAX Auto desk (Autodesk Inc.).

The following HLA-DRβ1* molecule's 3D structure previously determined by X-ray crystallography was modified by molecular dynamics according to the *Aotus* DR (AoDR-like) differences described by Suarez et al. [15]: HLA-DRβ1*0301 (PDB code Nr. 1A6A) [17], HLA-DRβ1*0401 (PDB code Nr. 1J8H) [18] and HLA-DRβ1*0101 (PDB code Nr. 1DLH) [19].

3. Result and discussion

3.1. Immunological studies

It has been thoroughly demonstrated that cHABP critical binding residues have to be specifically modified (mHABPs) to turn them into highly immunogenic and sterile immunity-inducing peptides [12–14,26,27] and that such aminoacid replacements modify their 3D structure (Fig. 1B), thereby inducing a change in their random coil structure, as in 4313 involving type III distorted β-turn in **10022.43**, or changing classical type III-turn in 4044 into type III distorted β-turn in **24112.39** or displacing the α-helical structure in 1585 five residues backwards in **10014.35**.

Such structural modifications have led to very clear biological differences allowing them to bind with high capacity to some allele molecules encoded by the HLA-DRβ1* region to induce high antibody titre production in *Aotus* monkeys, as assessed by IFA (Fig. 1C). The parasite's organelles from which these protein's aminoacid sequence were derived has also been recognised, i.e. micronemes and merozoite membrane recognised by AMA-1 protein anti-**10022.43** and merozoite membrane recognised by anti-**24112.39**- and **10014.35**-induced mHABP MSP-2 and MSP-1, respectively (Fig. 1C).

These antibodies also recognised molecules having the same molecular weight as the proteins from which their corresponding native amino acid sequence was derived, as assessed in some monkeys by WB (Fig. 1D) and thoroughly described elsewhere [12,26,29–31]. More importantly, they were able to induce sterile protective immunity against experimental intravenous challenge with 100,000 infected RBC freshly obtained from another *Aotus* infected with the highly-infective *Aotus*-adapted *P. falciparum* FVO strain (Fig. 1E). Controls and non-protected monkeys rapidly developed very high parasitaemia that required prompt treatment.

Such data, corroborated in hundreds of experiments [12,26], has clearly demonstrated that inducing sterile protective immunity requires cHABPs to be specifically modified into mHABPs, as has been thoroughly demonstrated.

Replacements in these peptides has altered their binding capacity to purified HLA-DRβ1* molecules while all cHABPs in this study did not bind to the purified class II molecules reported here but mHABPs did so. **10022** thus bound with ~42% capacity to the HLA-DRβ1*0701 molecule but also displayed HLA-DRβ1*0302 binding registers and motifs [26] which is why the latter was chosen for interaction studies. **24112** bound with high capacity (53%) to HLA-DRβ1*0401 [26] and **10014** bound to HLA-DRβ1*1101 (61%) but also displayed HLA-DRβ1*0101 binding motifs and registers [26], the latter thus being chosen for superimposition analysis (Fig. 1B).

The 3D structure of cHABPs and their corresponding mHABPs was analysed by ^{1}H NMR in the search for a logical and rational methodology for vaccine development to identify the physical–chemical rules determining their immunological outcome. This study thus endeavoured to identify further physical–chemical characteristics adding new rules or principles to previously determined ones [12,26,27].

Since distances are critical in these peptides' binding to HLA-DRβ1* molecules, the inter-atomic distances between the most distant atoms fitting into HLA-DRβ1* molecule pockets from 1 to 9 was determined, showing that **10022** had a 26.66 Å distance, **24112** had 26.84 Å and **10014** had 26.66 Å, thereby allowing them to fit perfectly into class II molecule's essential pockets (P1, P4, P6, P9) (Fig. 2A, D, G showing mHABP backbone atoms and peptide bonds planes).

3.2. Critical role of Φ and Ψ angles

The 3D structure of **10022.43** mHABP displayed two left-handed polyproline II-like helix ($PPII_L$) regions (Fig. 2B, highlighted in green), the first from position (p) one (p1) in the aminoacid sequence containing the HLA-DRβ1*0302 molecule binding motif and binding register spanning p1F to p4S (from this point onwards, amino acids will be referred to by their single-letter code), having −140.2° to −62.4° Φ ($PPII_L$ helix range: −93° ± 25°) and Ψ dihedral

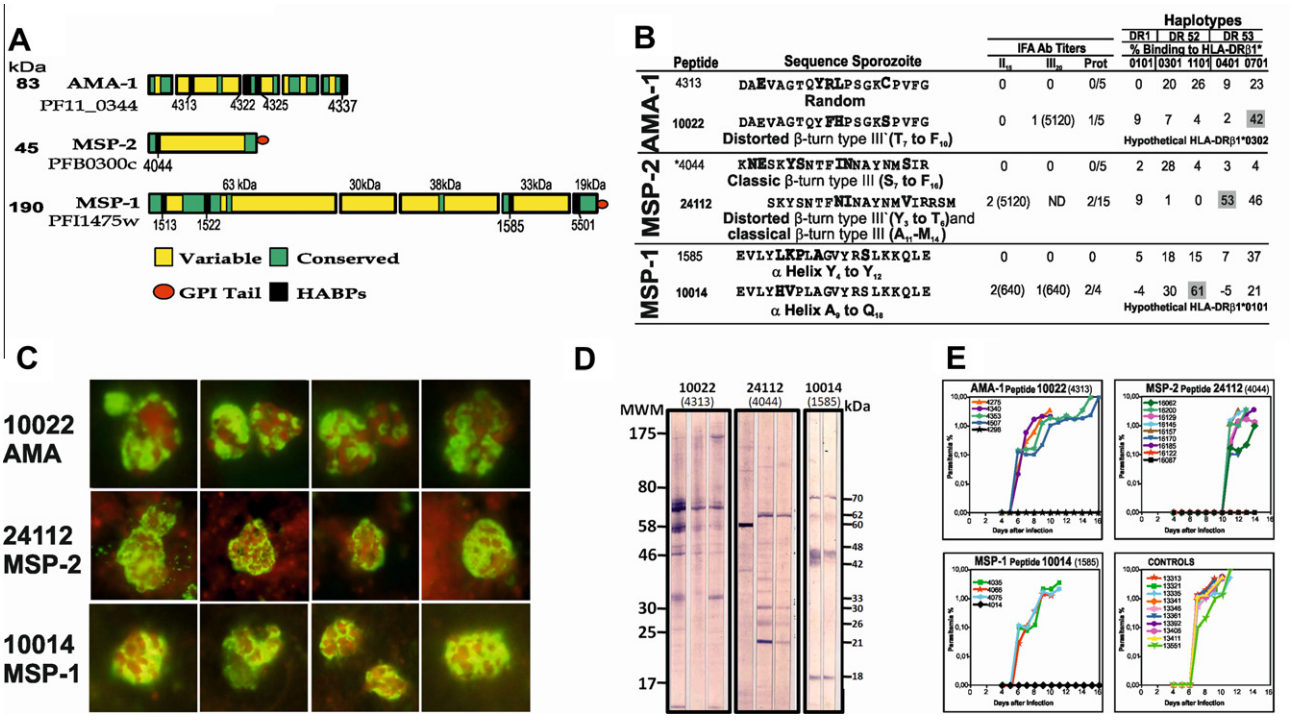


Fig. 1. (A) Schematic representation (approximate proportions) of the most relevant merozoite proteins (in bold) coloured according to the colour code given below, with their molecular weights (MW) in kDa and their accession numbers in the *P. falciparum* genome. The localisation of cHABPs (black bars) shown in this manuscript is given in enlarged numbers. (B) cHABP and mHABP amino acid sequence from AMA-1, MSP-2 and MSP-1 (CBR and amino acids are shown in bold), including each peptide's structural features elucidated by ^{1}H NMR. IFA Ab titre is the maximum sera dilution shown in parenthesis in the number of monkeys immunised with the corresponding peptide. II_{15} and III_{20} represent bleeding 15 or 20 days after 2nd (II) or 3rd (III) immunisation. Prot: total number of *Aotus* monkeys fully protected against intravenous challenge by freshly-obtained 100% infective *Aotus*-adapted *P. falciparum* FVO strain. HLA-DRβ1* binding capacity means peptide ability to compete with reference peptide and displace its binding capacity to the purified HLA-DRβ1* molecules; ~50% binding (shadowed) was considered highly positive. (C) IFA pattern recognised by *Aotus* sera in *P. falciparum* schizonts: anti-**10022** displayed a microneme (large dot) as well as a membrane fluorescence pattern; anti-**24112** and anti-**10014** displayed classical merozoite membrane reactivity. (D) Western blot (WB) analysis with mature schizont lysate displaying reactivity with molecules corresponding to the MW of the protein from which the cHABP aminoacid sequence was derived and their processing products. (E) The course of parasitaemia levels on a semi-logarithmic scale in monkeys immunised with corresponding peptides.

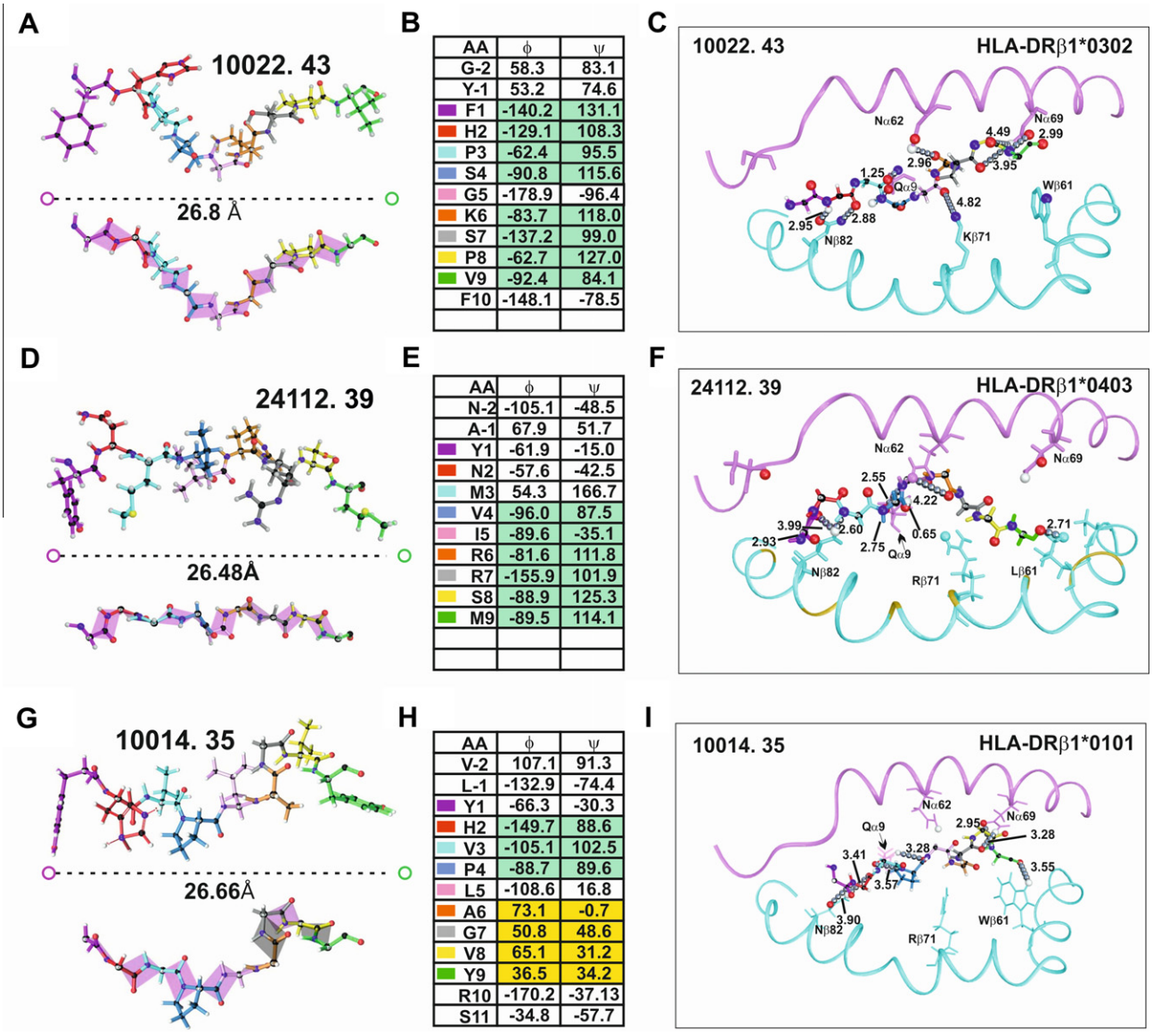
angles from +131.1° to 95.5° ($PPII_L$ helix range: +135° ± 15°) and a second $PPII_L$ from p6 K to p9 V having from −137.2° to −62.7° Φ angles and +127.0° to +84.1° Ψ angles (Fig. 2B). Some angles were slightly distorted in some regions, but very close to $PPII_L$ helix canonical structure formed by three residues per turn and 9.07 Å per pitch, and no H-bonds were established [33–35].

This conformation allowed **10022.43** mHABP 3D region (determined by ^{1}H-NMR) to almost fit perfectly into the HLA-DRβ1*0302 structure (determined by X-ray crystallography) (Fig. 2C). Although experimentally displaying high HLA-DRβ1*0701 and low HLA-DRβ1*0301 purified molecule binding capacity, it has to be kept in mind that while this allele displays the β86 V dimorphic sequence in pocket 1 in ~50% of humans (allowing large apolar residues like L, I and V to fit), >90% of HLA-DRβ1*03 molecules in *Aotus* monkeys display the β86G dimorphism in the same pocket, allowing aromatic residues such as F, W and Y, to fit, thereby allowing phenylalanine (F) to fit into P1 in *Aotus* monkeys. The other **10022** replacements in this region (Hp2L and Sp7C) concerned residues pointing away from this class II molecule's peptide-binding region (PBR), probably making contact with the TCR.

Superimposing **10022.43** onto the HLA-DRβ1*0302 molecule without any further refinements allowed the spontaneous formation of 6 H-bonds (⩽4.0 Å distance) and 2 van der Waals (vdW) interactions (⩽5.0 Å cut-off distance) (Fig. 2C and Table 1) where the Nβ82 HLA-DRβ1*0302 lateral chain atoms established a 9-membered ring bidentate H-bond [36] with p2H; this was similar to that established between Nα69 with p7S, p9 V and p8P, being quite similar to the canonical H-bonds established for HLA-DRβ1* molecules in the elegant work by Jardetsky et al. [37]. Qα9, Nα62, and Nβ71 established only one H-bond each with p4S, p6 K and p5G backbone atoms, respectively; the other bonds required for bidentate ring conformation between Qα9 and Nα62 had >5.0 Å and have not been displayed in Fig. 2C. This partial difference in distance could have been due to the superimposition of these two structures, as determined by two different methodologies (^{1}H NMR in solution for mHABP **10022.43** and X-ray crystallography for HLA-DRβ1*0301), or slight structural differences between *Aotus* HLA-DRβ1* molecules and human HLA-DRβ1* proteins.

Two consecutive $PPII_L$-like regions were identified in the **24112.39** conformer from p4 V to p9 M (Fig. 2E, highlighted in green), having Φ angles ranging from −155.9° to −81.6° and Ψ angles ranging from +125.3° to +87.5° ($PPII_L$ helix range as described above) with only p5I not completely fulfilling the Ψ angle range. This conformer led to establishing bidentate H-bonds with corresponding atoms from mHABP **24112.39** (Table 1) when superimposed on the HLA-DRβ1*0403 3D molecule, modified according to *Aotus* monkey's amino acid sequence. Replacing Lβ61W in the *Aotus* (yellow in the HLA-DRβ1*0403 blue β ribbon, Fig. 2F) involved molecular modelling with a conjugate gradient algorithm to minimise energy [15,16]; these differences in volume (166.7 Å^3 *cf* 227.8 Å^3) and surface (170 Å^2 versus 255 Å^2) could probably have accounted for this interaction's longer distance (4.22 Å).

mHABP **10014.35** bound to HLA-DRβ1*1101 with high capacity (61%) and very poorly to HLA-DRβ1*0101, displaying the binding



AA	ϕ	ψ
G-2	58.3	83.1
Y-1	53.2	74.6
F1	-140.2	131.1
H2	-129.1	108.3
P3	-62.4	95.5
S4	-90.8	115.6
G5	-178.9	-96.4
K6	-83.7	118.0
S7	-137.2	99.0
P8	-62.7	127.0
V9	-92.4	84.1
F10	-148.1	-78.5

AA	ϕ	ψ
N-2	-105.1	-48.5
A-1	67.9	51.7
Y1	-61.9	-15.0
N2	-57.6	-42.5
M3	54.3	166.7
V4	-96.0	87.5
I5	-89.6	-35.1
R6	-81.6	111.8
R7	-155.9	101.9
S8	-88.9	125.3
M9	-89.5	114.1

AA	ϕ	ψ
V-2	107.1	91.3
L-1	-132.9	-74.4
Y1	-66.3	-30.3
H2	-149.7	88.6
V3	-105.1	102.5
P4	-88.7	89.6
L5	-108.6	16.8
A6	73.1	-0.7
G7	50.8	48.6
V8	65.1	31.2
Y9	36.5	34.2
R10	-170.2	-37.13
S11	-34.8	-57.7

Fig. 2. Left panel: A, D and G, AMA-1-, MSP-2- and MSP-1-derived mHABPs displaying their lowest energy conformer 3D structure (respectively) as determined by ^{1}H NMR, named according to our institute's serial number followed by a dot, and their Angstrom distance (Å) between the most distant atoms fitting into pockets 1–9 of HLA-DRβ1* molecules. Central panel: B, E and H corresponding conformer (Φ and Ψ) dihedral angle measurements, highlighting the PPII$_L$ (green) and the αL (yellow) regions. Right panel: Superimposition of mHABP backbone 3D structure (assessed by ^{1}H NMR) onto HLA-DRβ1* molecules (determined by X-ray crystallography) displaying the H-bonds established between the peptide's backbone and HLA-DRβ1* lateral chain atoms measured in Angstroms (Å). (C) Top view of **10022.43** superimposed onto HLA-DRβ1*0302 (modified from PDB code 1A6A) (F) **24112.39** superimposed onto HLA-DRβ1*0401 (PDB code 1J8H) I) **10014.35** superimposed on HLA-DRβ1*0101 (modified from PDB code 1DLH). β-chain is shown in pink ribbon and β-chain in pale blue ribbon. H-bonds are shown in silver chains with their 'corresponding distances.

motifs and binding registers characteristic for these two human alleles, only the binding registers being displaced four residues upstream for HLA-DRβ1*0101, as has been described by others [38]. However, it must be born in mind that this allele has very low frequency in such monkeys (<5% *Aotus* population); identifying the few mHABPs displaying this binding characteristic is thus of prime importance.

10014.35 displayed a typical PPII$_L$ region spanning from p2H to p4P with characteristic Φ and Ψ torsion angles from −149.7° to −88.7° for the former and +102.5° to +88.6° for the latter, three residues being involved in this structure. This mHABP displayed a short left-handed α-conformation (αL) from p7G tp p9Y as described [33–35], having Φ angles +65.1° to +36.5° and +48.6° to +31.2° for Ψ (Fig. 2H, shown in yellow), being close to this region's values (+60.7° Φ, +52.4° Ψ and ⩾4 residues) [39], suggesting that peptides having dihedral torsion angles different to PPII$_L$ could also participate in peptide-MHCII complex formation (Fig. 2H).

When **10014.35** was superimposed it spontaneously established 6 H-bonds and 1 vdW interaction with the HLA-DRβ1*0101 molecule (Fig. 2I and Table 1) via three canonical bidentate H-bonds between Nβ82 lateral chain atoms and p2H backbone atoms, Qα9 and p4N and Nα69 and p7G and p9Y. One single H-bond was established between Wβ61 and p9Y, as classically defined. The distance between Nα62 and p6A was too large to be considered as a stabilising interaction, but no molecular modifications were performed in the human HLA-DRβ1*0101 molecule according to *Aotus*–DR-like differences, due to several aminoacid differences in the HLA-DRβ1*β chain.

The impact of Φ and Ψ dihedral angles on the appropriate orientation of mHABP backbone atoms and their 3D conformations to

Table 1
Distances measurements are given in Angstroms (Å) for mHABP backbone atoms and their corresponding HLA-DRβ1* lateral chain atoms involved in H-bonds or vdW interactions.

	P1	P9
A. DAEVAGTQYFHPSGKSPVFG		
HLA-DRβ1∗0302	**10022.43**	Distance Å
Nβ82:Oδ1	HN:H2	2.95
Nβ82:Hδ22	O:H2	2.88
Qα9:Oε1	HN:S4	1.25
Nα62:HNδ2	O:K6	2.96
Nα69:HNδ2	O:P8	4.49
Nα69:HNδ1	O:S7	3.95
Nα69:Oδ1	HN:V9	2.99
Nβ71:HH21	O:G5	4.82
B. SKYSNTFNINAYNMVIRRSM		
HLA-DRβ1∗0403	**24112.39**	Distance Å
Nβ82:Oδ1	HN:N2	2.75
Nβ82:Hδ21	O:N2	3.99
Nβ82:Hδ21	O:Y1	2.93
Qα9:Oε1	HN:V4	2.55
Qα9:Hε21	O:V4	0.65
Nα62:Hδ21	O:R6	4.22
Lα61:Hδ12	O:M9	2.71
C. EVLYHVPLAGVYRSLKKQLE		
HLA-DRβ1∗0101	**10014.35**	Distance Å
Nβ82:Oδ1	HN:H2	3.90
Nβ82:Hδ22	O:H2	3.41
Qα9:Hε22	N:P4	3.57
Qα9:Hε21	O:P4	3.28
Nα69:Hδ22	O:G7	3.28
Nα69:Oδ1	HN:Y9	2.95
Wβ61:Hε1	O:Y9	3.55

allow the spontaneous formation of 7–8 canonical H-bonds and vdW interactions with HLA-DRβ1* lateral chain atoms can be clearly seen. This would partly explain the **high immunogenicity and protective immunity** induced by these mHABPs, suggesting that such structural characteristics form the basis for new rules or principles required for **sterile immunity to become induced** and, when taken together with the previously published ones, are fundamental for vaccine development [11–14,26,27], malaria being one of them.

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References

[1] R.L. Coppel, Vaccinating with the genome: a Sisyphean task?, Trends Parasitol 25 (2009) 205–212.
[2] R.F. Anders, The case for a subunit vaccine against malaria, Trends Parasitol. 27 (2011) 330–334.
[3] L. Schwartz, G.V. Brown, B. Genton, V.S. Moorthy, A review of malaria vaccine clinical projects based on the WHO rainbow table, Malar J. 11 (2012) 11.
[4] J.S. Richards, J.G. Beeson, The future for blood-stage vaccines against malaria, Immunol. Cell Biol. 87 (2009) 377–390.
[5] C.J. Murray, L.C. Rosenfeld, S.S. Lim, K.G. Andrews, K.J. Foreman, D. Haring, N. Fullman, M. Naghavi, R. Lozano, A.D. Lopez, Global malaria mortality between 1980 and 2010: a systematic analysis, Lancet 379 (2012) (1980) 413–431.
[6] M.E. Patarroyo, P. Romero, M.L. Torres, P. Clavijo, A. Moreno, A. Martinez, R. Rodriguez, F. Guzman, E. Cabezas, Induction of protective immunity against experimental infection with malaria using synthetic peptides, Nature 328 (1987) 629–632.
[7] M.E. Patarroyo, R. Amador, P. Clavijo, A. Moreno, F. Guzman, P. Romero, R. Tascon, A. Franco, L.A. Murillo, G. Ponton, et al., A synthetic vaccine protects humans against challenge with asexual blood stages of Plasmodium falciparum malaria, Nature 332 (1988) 158–161.
[8] R. Amador, A. Moreno, L.A. Murillo, O. Sierra, D. Saavedra, M. Rojas, A.L. Mora, C.L. Rocha, F. Alvarado, J.C. Falla, et al., Safety and immunogenicity of the synthetic malaria vaccine SPf66 in a large field trial, J. Infect. Dis. 166 (1992) 139–144.
[9] M.V. Valero, L.R. Amador, C. Galindo, J. Figueroa, M.S. Bello, L.A. Murillo, A.L. Mora, G. Patarroyo, C.L. Rocha, M. Rojas, et al., Vaccination with SPf66, a chemically synthesised vaccine, against Plasmodium falciparum malaria in Colombia, Lancet 341 (1993) 705–710.
[10] A. Bermudez, C. Reyes, F. Guzman, M. Vanegas, J. Rosas, R. Amador, R. Rodriguez, M.A. Patarroyo, M.E. Patarroyo, Synthetic vaccine update: applying lessons learned from recent SPf66 malarial vaccine physicochemical, structural and immunological characterization, Vaccine 25 (2007) 4487–4501.
[11] L.E. Rodriguez, H. Curtidor, M. Urquiza, G. Cifuentes, C. Reyes, M.E. Patarroyo, Intimate molecular interactions of P. falciparum merozoite proteins involved in invasion of red blood cells and their implications for vaccine design, Chem. Rev. 108 (2008) 3656–3705.
[12] M.E. Patarroyo, M.A. Patarroyo, Emerging rules for subunit-based, multiantigenic, multistage chemically synthesized vaccines, Acc. Chem. Res. 41 (2008) 377–386.
[13] M.E. Patarroyo, H. Almonacid, A. Moreno-Vranich, The role of amino acid electron-donor/acceptor atoms in host-cell binding peptides is associated with their 3D structure and HLA-binding capacity in sterile malarial immunity induction, Biochem. Biophys. Res. Commun. 417 (2012) 938–944.
[14] M.E. Patarroyo, G. Cifuentes, C. Pirajan, A. Moreno-Vranich, M. Vanegas, Atomic evidence that modification of H-bonds established with amino acids critical for host-cell binding induces sterile immunity against malaria, Biochem. Biophys. Res. Commun. 394 (2010) 529–535.
[15] C.F. Suarez, M.E. Patarroyo, E. Trujillo, M. Estupinan, J.E. Baquero, C. Parra, R. Rodriguez, Owl monkey MHC-DRB exon 2 reveals high similarity with several HLA-DRB lineages, Immunogenetics 58 (2006) 542–558.
[16] M.A. Patarroyo, A. Bermudez, C. Lopez, G. Yepes, M.E. Patarroyo, 3D analysis of the TCR/pMHCII complex formation in monkeys vaccinated with the first peptide inducing sterilizing immunity against human malaria, PLoS One 5 (2010) e9771.
[17] P. Ghosh, M. Amaya, E. Mellins, D.C. Wiley, The structure of an intermediate in class II MHC maturation: CLIP bound to HLA-DR3, Nature 378 (1995) 457–462.
[18] J. Hennecke, D.C. Wiley, Structure of a complex of the human alpha/beta T cell receptor (TCR) HA1.7, influenza hemagglutinin peptide, and major histocompatibility complex class II molecule, HLA-DR4 (DRA∗0101 and DRB1∗0401): insight into TCR cross-restriction and alloreactivity, J. Exp. Med. 195 (2002) 571–581.
[19] L.J. Stern, J.H. Brown, T.S. Jardetzky, J.C. Gorga, R.G. Urban, J.L. Strominger, D.C. Wiley, Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide, Nature 368 (1994) 215–221.
[20] K.J. Smith, J. Pyrdol, L. Gauthier, D.C. Wiley, K.W. Wucherpfennig, Crystal structure of HLA-DR2 (DRA∗0101, DRB1∗1501) complexed with a peptide from human myelin basic protein, J. Exp. Med. 188 (1998) 1511–1520.
[21] K.C. Garcia, M. Degano, R.L. Stanfield, A. Brunmark, M.R. Jackson, P.A. Peterson, L. Teyton, I.A. Wilson, An alphabeta T cell receptor structure at 25 A and its orientation in the TCR-MHC complex, Science 274 (1996) 209–219.
[22] K.C. Garcia, M. Degano, L.R. Pease, M. Huang, P.A. Peterson, L. Teyton, I.A. Wilson, Structural basis of plasticity in T cell receptor recognition of a self peptide-MHC antigen, Science 279 (1998) 1166–1172.
[23] M.G. Rudolph, I.A. Wilson, The specificity of TCR/pMHC interaction, Curr. Opin. Immunol. 14 (2002) 52–65.
[24] P. Marrack, J.P. Scott-Browne, S. Dai, L. Gapin, J.W. Kappler, Evolutionarily conserved amino acids that control TCR-MHC interaction, Annu. Rev. Immunol. 26 (2008) 171–203.
[25] S. Dai, E.S. Huseby, K. Rubtsova, J. Scott-Browne, F. Crawford, W.A. Macdonald, P. Marrack, J.W. Kappler, Crossreactive T Cells spotlight the germline rules for alphabeta T cell-receptor interactions with MHC molecules, Immunity 28 (2008) 324–334.
[26] M.E. Patarroyo, A. Bermudez, M.A. Patarroyo, Structural and immunological principles leading to chemically synthesized, multiantigenic, multistage, minimal subunit-based vaccine development, Chem. Rev. 111 (2011) 3459–3507.
[27] A. Moreno-Vranich, M.E. Patarroyo, Steric-electronic effects in malarial peptides inducing sterile immunity, Biochem. Biophys. Res. Commun. 423 (2012) 857–862.
[28] R.B. Merrifield, Solid phase peptide synthesis. I. The synthesis of a tetrapeptide, J. Am. Chem. Soc. 8 (5) (1963) 2149.
[29] G. Cifuentes, M.E. Patarroyo, M. Urquiza, L.E. Ramirez, C. Reyes, R. Rodriguez, Distorting malaria peptide backbone structure to enable fitting into MHC class II molecules renders modified peptides immunogenic and protective, J. Med. Chem. 46 (2003) 2250–2253.
[30] F. Espejo, M. Cubillos, L.M. Salazar, F. Guzman, M. Urquiza, M. Ocampo, Y. Silva, R. Rodriguez, E. Lioy, M.E. Patarroyo, Structure, immunogenicity, and protectivity relationship for the 1585 malarial peptide and its substitution analogues, Angew. Chem. Int. Ed. Engl. 40 (2001) 4654–4657.
[31] J. Purmova, L.M. Salazar, F. Espejo, M.H. Torres, M. Cubillos, E. Torres, Y. Lopez, R. Rodriguez, M.E. Patarroyo, NMR structure of *Plasmodium falciparum* malaria peptide correlates with protective immunity, Biochim. Biophys. Acta 1571 (2002) 27–33.
[32] H.G. Rammensee, T. Friede, S. Stevanoviic, MHC ligands and peptide motifs: first listing, Immunogenetics 41 (1995) 178–228.
[33] W.G. Han, J. Jalkanen, M. Elstener, S. Suhai, Theoretical study of aqueous N-acetyl-l-alanine N′-methylamide: structures and Raman, VCD, and ROA spectra, J. Phys. Chem. B 102 (1998) 2587–2602.

[34] A. Rath, A.R. Davidson, C.M. Deber, The structure of "unstructured" regions in peptides and proteins: role of the polyproline II helix in protein folding and recognition, Biopolymers 80 (2005) 179–185.
[35] M. Kuemin, S. Schweizer, C. Ochsenfeld, H. Wennemers, Effects of terminal functional groups on the stability of the polyproline II structure: a combined experimental and theoretical study, J. Am. Chem. Soc. 131 (2009) 15474–15482.
[36] A.A. Adzhubei, M.J. Sternberg, Left-handed polyproline II helices commonly occur in globular proteins, J. Mol. Biol. 229 (1993) 472–493.
[37] T.S. Jardetzky, J.H. Brown, J.C. Gorga, L.J. Stern, R.G. Urban, J.L. Strominger, D.C. Wiley, Crystallographic analysis of endogenous peptides associated with HLA-DR1 suggests a common polyproline II-like conformation for bound peptides, Proc. Natl. Acad. Sci. USA 93 (1996) 734–738.
[38] Y. Li, H. Li, R. Martin, R.A. Mariuzza, Structural basis for the binding of an immunodominant peptide from myelin basic protein in different registers by two HLA-DR2 proteins, J. Mol. Biol. 304 (2000) 177–188.
[39] M. Novotny, G.J. Kleywegt, A survey of left-handed helices in protein structures, J. Mol. Biol. 347 (2005) 231–241.