Biochemical and Biophysical Research Communications 377 (2008) 1279–1283

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Identification and evaluation of universal epitopes in *Plasmodium vivax* Duffy binding protein

Carolina Saravia [a,b], Paola Martinez [a], Diana S. Granados [a,c], Carolina Lopez [a,c], Claudia Reyes [a], Manuel A. Patarroyo [a,d,*]

[a] *Fundación Instituto de Inmunología de Colombia, Molecular Biology, Carrera 50#26-20, Bogota, Colombia*
[b] *Universidad de Los Andes, Bogota, Colombia*
[c] *Universidad Nacional de Colombia, Bogota, Colombia*
[d] *Universidad del Rosario, Bogota, Colombia*

ARTICLE INFO

Article history:
Received 26 October 2008
Available online 8 November 2008

Keywords:
Malaria
Plasmodium vivax
Duffy binding protein (DBP)
Universal epitopes

ABSTRACT

Selected *Pv*DBP-derived synthetic peptides were tested in competition assays with HLA molecules in order to identify and evaluate their binding to a wide range of MHC class II molecules. Binding was evaluated as the peptide's ability to displace the biotinylated control peptide ($HA_{306-318}$) and was detected by a conventional ELISA. Thus, one epitope for the HLA-DR1 molecule, two epitopes for the HLA-DR4 molecule, six epitopes for the HLA-DR7 molecule and three epitopes for the HLA-DR11 molecule displaying a high binding percentage (above 50%) were experimentally obtained. The *in vitro* results were compared with the epitope prediction results. Two peptides behaved as universal epitopes since they bound to a larger number of HLA-DR molecules. Given that these peptides are located in the conserved *Pv*DBP region II, they could be considered good candidates to be included in the design of a synthetic vaccine against *Plasmodium vivax* malaria.

Malaria is one of the most widely distributed infectious diseases around the world. It is caused by obligated intracellular parasites of the genus *Plasmodium*, being *Plasmodium vivax* and *Plasmodium falciparum* the two most prevalent species [1]. Even though *P. vivax* is one of the main pathogens causing malaria and produces 70–80 million annual cases [2–4], basic research on the biological, cellular, and molecular aspects of this species has been largely hampered by the impossibility of growing this parasite in continuous *in vitro* culture [5].

The parasite possesses a complex life cycle with repetitive parasitic growth cycles and burst of host's red blood cells (RBCs). During *P. vivax* invasion to RBCs, blood-stage forms (namely merozoites) use a wide range of proteins that includes both surface and apical-organelle proteins (contained inside rhoptries, micronemes, and dense granules) for recognizing and invading new RBCs, which is why most vaccine development research has focused on these important targets [6,7].

Among the most promising vaccine targets is the *P. vivax* Duffy binding protein (*Pv*DBP), which is a 140 kDa microneme-secreted protein [6] that mediates the merozoite-RBC irreversible attachment and junction formation by interacting with the Duffy antigen receptor for chemokines (DARC) expressed on RBCs [7]. Its amino-terminal cysteine-rich region (denoted as region II or *Pv*DBP region II) contains about 330 amino acids and plays an important role in receptor's recognition [8–10] despite being highly polymorphic [2,11,12].

Various strategies have been employed in order to define and characterize potentially important vaccine candidate antigens against *Plasmodium* spp. Among such strategies, the mapping of universal epitopes (epitopes binding to a wide range of human leukocyte antigen (HLA) alleles) and the assessment of molecule or molecule combinations capable of raising a protective immune response against the pathogen, constitute useful alternatives for defining suitable subunits to be included in the design of a multi-antigenic, subunit-based antimalarial vaccine.

In this study, *Pv*DBP peptides belonging to the functionally important region II were assayed for their ability to bind several HLA class II molecules in order to define universal epitopes that could aid to accomplish the above mentioned goal for *P. vivax*.

Materials and methods

Peptides. The peptides previously reported by Ocampo et al. [10] on *Pv*DBP were assessed in this study and corresponded to peptides 1623, 1625, 1627, 1631, 1635, 1637, 1638, 1639, 1642, and 1660. The peptide's amino acid sequences are shown in Table 1

* Corresponding author. Address: Fundación Instituto de Inmunología de Colombia, Molecular Biology, Carrera 50#26-20, Bogota, Colombia. Fax: +57 1 4815269.
E-mail address: mapatarr@fidic.org.co (M.A. Patarroyo).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.10.153

in a one letter code. Synthetic peptides were synthesized by the multiple-solid-phase technique, using tert-butoxycarbonyl (t-Boc) strategy as previously described [12]. They were then purified by reverse-phase HPLC on a C18 LiChrospher column (Merck, Germany). The products were assessed by analytical HPLC and MALDI-TOF-MS. The biotinylated $HA_{306–318}$ (PKYVKQNTLKLAT) control peptide used for HLA binding and competition experiments was synthesized by using sulfo-NHS-LC-Biotin (Pierce Chemical, Rockford). This biotin derivative incorporates a six carbon-spacer long chain to increase the distance between the biotin moiety and the peptide. Biotin was coupled by following the standard solid-phase peptide synthesis coupling procedure; coupling efficacy was evaluated by the Kaiser test and standard ELISA.

HLA-DR molecule purification. Human HLA-DR molecules were purified from DR1, WT100BIS (DRB1*0101); DR4, BSM (DRB1*0401); DR7, EKR (DRB1*0701); and DR11, 9043 (DRB1*1101) homozygous EBV-B cell lysates by affinity chromatography [13] using anti-HLA-DR mAb L-243 crosslinked to protein A–Sepharose CL-4B (Amersham–Pharmacia Biotech AB) as affinity support. Cells were lysed at 108 cells mL^{-1} density on ice for 60 min in 1% (v/v) Nonidet P-40, 25 mM iodoacetamide, 1 mM phenylmethylsulfonyl fluoride, and 10 μg mL^{-1} of each of the following: soybean trypsin inhibitor, antipain, pepstatin, leupeptin and chymostatin in 0.05 M sodium phosphate buffer, and 0.15 M NaCl, pH 7.5. Nucleic acids and debris were cleared from lysates by spinning at 27,000*g* for 30 min. After 0.2 volumes of 5% sodium deoxycholate were added to the supernatant and mixed for 10 min, the lysate was centrifuged at 100,000*g* for 2 h and subsequently filtered through a 0.45 μm membrane. Lysates were first passed through a Sepharose CL-4B pre-column and subsequently over the Sepharose A-mAb L-243 protein column for class II molecule affinity purification. The affinity column was then washed with: (i) 20 column volumes of 50 mM Tris–HCl, pH 8.0, 0.15 M NaCl, 0.5% NP-40, and 0.5% DOC; (ii) 5 column volumes of 50 mM Tris–HCl, pH 9.0, 0.5 M NaCl, 0.5% NP-40, and 0.5% sodium deoxycholate; and (iii) 5 column volumes of 2 mM Tris–HCl, pH 8.0, 1% octyl-β-D-glucopyranoside (Sigma, St. Louis). HLA-DR molecules were eluted with 0.05 M diethylamine in 0.15 M NaCl, pH 11.5, containing 1% octyl-β-D-glucopyranoside and 1 mM EDTA. The eluate was immediately neutralized with 1/20 volume of 1 M Tris–HCl, pH 6.8. Preparations were kept in aliquots at −70 °C until use.

Peptide-binding competition assays. Peptide-binding competition assays were conducted to measure unlabeled peptide ability to compete with the biotinylated control peptide (HA) for binding to purified HLA-DR molecules. Unlabeled peptides were used in excess, as described by other authors for direct binding assays [14,15]. A Multiskan ELISA reader (Labsystems, Franklin, MS) with 405 nm filter was used for determining peptide binding to HLA-DR molecules by measuring the optical densities (OD) in the presence vs. absence of competitor peptide. Inhibition was calculated as a percentage by using the formula: 100 × [1 − ΔOD in the presence of competitor/ΔOD in the absence of competitor peptide]. According to this assay, a good competitor was a peptide which was capable of inhibiting more than 50% of the indicator peptide's binding to the HLA molecule being tested.

IC_{50} values. The IC_{50} (50% inhibitory concentration) values of the selected peptides were calculated as the concentration of competitor peptide (*Pv*DBP peptide) needed to inhibit binding of biotinylated control peptide by 50% as described above, but varying the concentration of selected peptide. Peptide 1623 was used as negative control as it bound loosely to all molecules (data not shown). The data obtained from IC_{50} values were normalized prior calculating their mean and standard deviation and used for graphing concentration vs. absorbance (data not shown).

In silico epitope prediction. The SYFPEITHI prediction tool (www.syfpeithi.de) was used in this study for determining possible anchoring residues to a particular class II HLA-DR molecule. This tool was developed by Rammensee et al. [16], based on previously published databases [17] as a suitable algorithm for predicting peptide binding to HLA-DR molecules in proteins such as p53. Once binding of the selected *Pv*DBP peptides to purified HLA-DR molecules was predicted using this tool, the results were compared to experimental data obtained from HLA–peptide-binding *in vitro* assays.

Sequence alignments. Peptide sequences previously reported by Ocampo et al. [10] were compared to the *Pv*DBP reported sequences available at GenBank (http://www.ncbi.nlm.nih.gov/Genbank/) by multiple sequence alignment using the ClustalW tool (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html) in order to determine whether or not these sequences are conserved among the different *P. vivax* isolates (GenBank accessions used were: AAZ81536, AAZ81535, AAZ81532, AAZ81528, AAZ81530, ABA39300, AAZ81531, ABA39301, AAZ81525, AAZ81527, AAZ81534, AAZ81533, AAZ81529, and AAZ81526).

Structure prediction by homology. The GENO3D server (http://geno3d-pbil.ibcp.fr/cgi-bin/geno3d_automat.pl?page=/GENO3D/geno3d_home.html) was used for developing a *P. vivax* DBP region II (Sal-I strain) homology-based structure prediction model where the 3D structure of *Plasmodium knowlesi* DBP region II (PDB 2C6J) was used as template. The polymorphic residues (obtained by sequence alignment) as well as the universal epitopes here found are shown in the predicted structure (Fig. 3).

Results

PvDBP peptides' binding to HLA-DRB1 alleles*

According to the results (Fig. 2), one peptide bound to the DRB1*0101 allele (peptide 1635 with a 66% binding percentage) (Fig. 2A), two to the DRB1*0401 allele: peptides 1635 (71%) and 1638 (72%) (Fig. 2B), six to the DRB1*0701 allele: peptides 1631 (76%), 1635 (54%), 1637 (60%), 1638 (99%), 1639 (97%), and 1642 (65%) (Fig. 2C) and three to the DRB1*1101 allele: peptides 1631 (52%), 1637 (78%), and 1638 (68%) (Fig. 2D). Additionally, preliminary binding studies found seven peptides (1631, 1635, 1637, 1638, 1639, 1642, and 1660) binding to the HLA-DRB1*1301 allele (data not shown).

1635 and 1638 are universal epitopes

As shown in Fig. 2, the results obtained experimentally evidenced that peptides 1635 and 1638 bound to a larger number of HLA-DRB1*

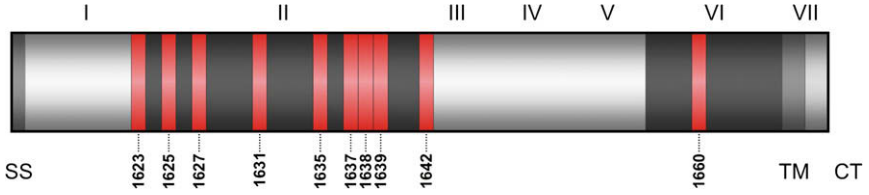

Fig. 1. Schematic localization of the peptides assessed in this study on *Pv*DBP. Each red band corresponds to one of the *Pv*DBP peptides assessed in this study. It can be seen that most peptides are located towards the protein's region II. SS, signal sequence; TM, transmembrane; CT, cytoplasmatic tail.

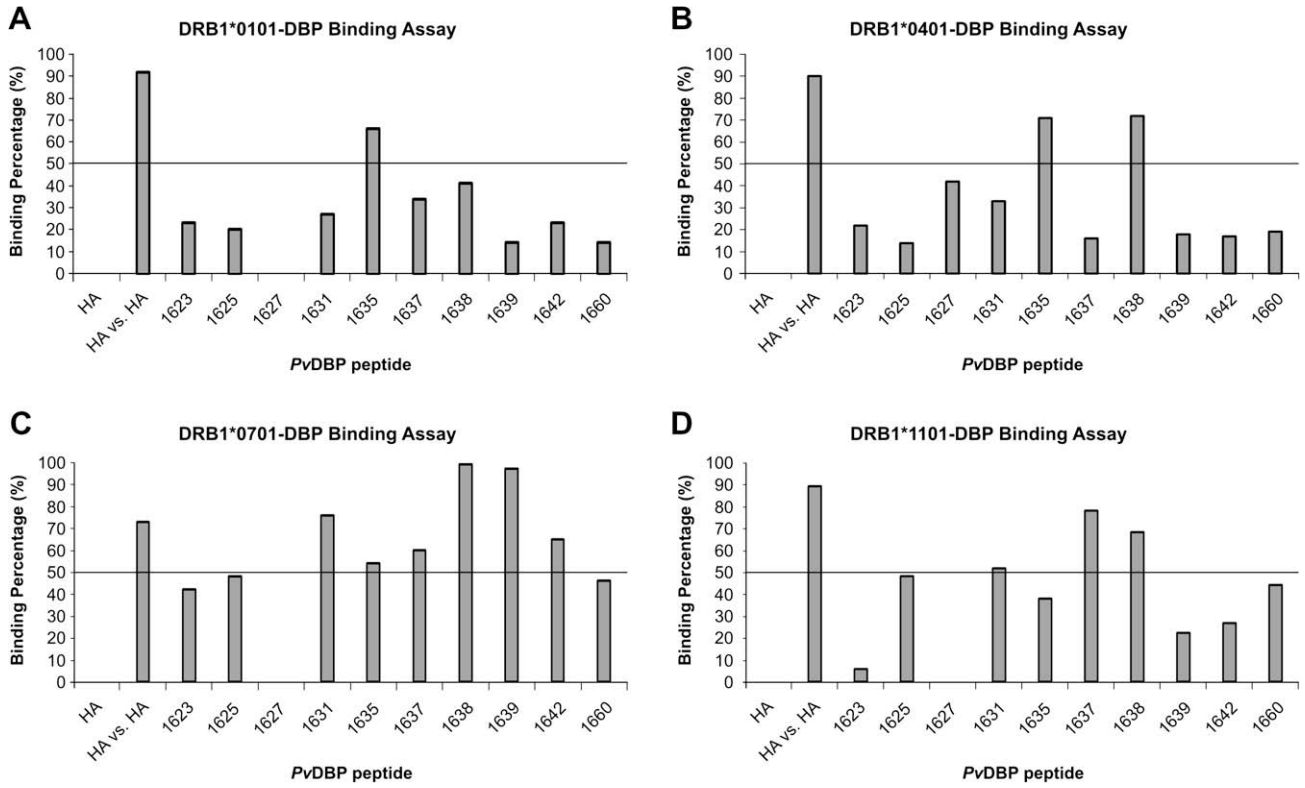

Fig. 2. Binding percentages of *Pv*DBP peptides to HLA-DBR1* alleles. A binding percentage above the horizontal line set at 50% binding determines that a peptide binds positively to the assessed HLA-DR molecule.

alleles, thereby being considered universal epitopes [18]. According to the IC_{50} values shown in Table 2, the strongest interaction occurred between both peptides 1635 and 1638 and the HLA-DRB1*0401 molecule (75.19 μM and 64.51 μM, respectively).

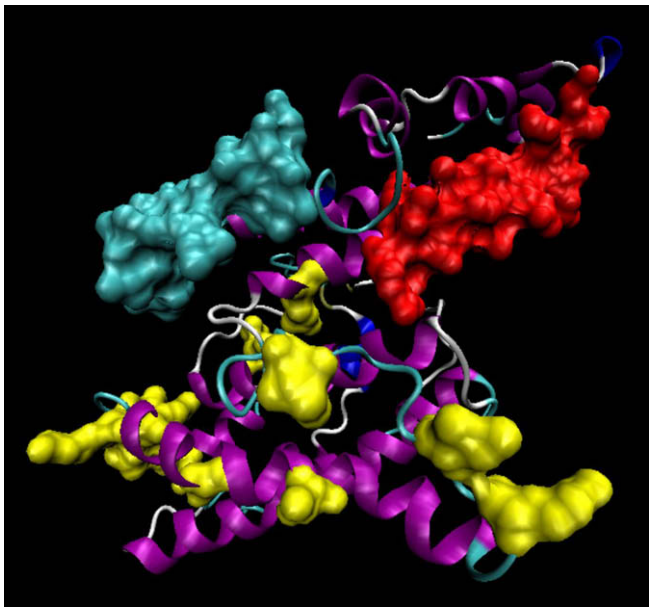

Fig. 3. Homology-based structure prediction model of the *Pv*DBP region II. The image shows the localization of polymorphic residues opposed to the localization of binding regions. Peptide 1635 (red) and 1638 (cyan) belonging to the conserved DARC binding region are distinctly located towards the upper section, whereas polymorphic residues (yellow) are markedly located in the lower section of *Pv*DBP region II. Graphics were developed using Visual Molecular Dynamics (VMD), version 1.8.6.

Epitope prediction

The SYFPEITHI tool was used for establishing the theoretical binding of the ten *Pv*DBP peptides to HLA DRB1*0101, DRB1*0401, DRB1*0701, and DRB1*1101 molecules, taking into account only peptides with a score above 25. These results were subsequently compared to the experimentally obtained peptides shown in Fig. 2.

According to the SYFPEITHI prediction, peptides 1631 (**G**IG**Y**S**K**VV**E**NNLRSI) and 1642 (**S**QP**V**D**S**SK**A**EKVPGD bind to the HLA-DRB1*0101 allele with a 26 and 25 score, respectively), while peptide 1635 binds to the HLA-DRB1*0401 allele with two different registers (**R**DY**V**S**EL**P**T**EVQKLK and **V**SE**L**P**TE**V**Q**KLKEKC, each with a 26 score). The program reported no peptides binding to the HLA-DRB1*0701 and HLA-DRB1*1101 alleles. The amino acids corresponding to positions P1, P4, P6, and P9 (and P7 in some cases) [19] were outlined by SYFPEITHI in bold (Table 3) since they corresponded to residues fitting into the main HLA-DRB1 allele's pockets on the peptide-binding region.

The prediction results suggest that the important amino acids for peptide 1631 anchoring into the HLA-DR1 molecule are: Gly-

Table 1
Peptide sequences.

Peptide	*Pv*DBP sequence
1623	SNGQPAGTLDNVLEFVTGHE
1625	TISSAIINHAFLQNTVMKNC
1627	IPDRRYQLCMKELTNLVNNT
1631	DMEGIGYSKVVENNLRSIFG
1635	RDYVSELPTEVQKLKEKCDG
1637	SYDQWITRKKNQWDVLSNKF
1638	ISVKNAEKVQTAGIVTPYDI
1639	LKQELDEFNEVAFENEINKR
1642	SQPVDSSKAEKVPGDSTHGN
1660	LNSNNNLSNGKLDIKEYKYR

Table 2
IC_{50} values and μM concentrations for peptides 1635 and 1638 with each of the HLA-DRB1* alleles.

Peptide	HLA-DRB1* alleles	Sequence (amino acid position)	IC_{50}	μM
		408 427		
1635	HLA-DRB1*0101	RDYVSELPTEVQKLKEKCDG	3910.34	130.34
1635	HLA-DRB1*0401	RDYVSELPTEVQKLKEKCDG	2255.77	75.19
1635	HLA-DRB1*0701	RDYVSELPTEVQKLKEKCDG	5968.75	198.96
		469 488		
1638	HLA-DRB1*0401	ISVKNAEKVQTAGIVTPYDI	1935.21	64.51
1638	HLA-DRB1*0701	ISVKNAEKVQTAGIVTPYDI	2347.83	78.26
1638	HLA-DRB1*1101	ISVKNAEKVQTAGIVTPYDI	4336.54	144.55

Table 3
SYFPEITHI-predicted peptides binding to the HLA-DRB1*0101 (A) and HLA-DRB1*0401 molecules (B).

Peptide	PvDBP sequence	Predicted epitope	Score
*(A) SYFPEITHI-predicted PvDBP peptides binding to HLA-DRB1*0101*			
1623	SNGQPAGTLDNVLEFVTGHE		
1625	TISSAIINHAFLQNTVMKNC		
1627	IPDRRYQLCMKELTNLVNNT		
1631	DMEGIGYSKVVENNLRSIFG	**G**IG**Y**S**K**VV**E**NNLRSI	26
1635	RDYVSELPTEVQKLKEKCDG		
1637	SYDQWITRKKNQWDVLSNKF		
1638	ISVKNAEKVQTAGIVTPYDI		
1639	LKQELDEFNEVAFENEINKR		
1642	SQPVDSSKAEKVPGDSTHGN	**S**QP**V**D**S**SK**A**EKVPGD	25
1660	LNSNNNLSNGKLDIKEYKYR		
*(B) SYFPEITHI-predicted PvDBP peptides binding to HLA-DRB1*0401*			
1623	SNGQPAGTLDNVLEFVTGHE		
1625	TISSAIINHAFLQNTVMKNC		
1627	IPDRRYQLCMKELTNLVNNT		
1631	DMEGIGYSKVVENNLRSIFG		
1635	RDYVSELPTEVQKLKEKCDG	**R**DY**V**S**E**LP**T**EVQKLK	26
		VSE**L**P**T**EV**Q**KLKEKC	26
1637	SYDQWITRKKNQWDVLSNKF		
1638	ISVKNAEKVQTAGIVTPYDI		
1639	LKQELDEFNEVAFENEINKR		
1642	SQPVDSSKAEKVPGDSTHGN		
1660	LNSNNNLSNGKLDIKEYKYR		

cine, Tyrosine, Lysine, and Valine, while for peptide 1642 are: Serine, Valine, Serine, and Alanine. For peptide 1635, SYFPEITHI predicted two binding epitopes to the HLA-DR4 molecule. For the first epitope, amino acids corresponding to P1, P4, P6, and P9 are: Arginine, Valine, Glutamic acid, and Threonine. For the second epitope, the binding amino acids are: Valine, Leucine, Threonine, and Glutamine, the latter one being polymorphic (Glutamine is switched to Arginine in some sequences). Binding prediction is not altered when the polymorphic residue is changed.

Sequence alignment

Since sequence conservation is a desired feature in the selection of vaccine candidates, the sequences available at GenBank for *Pv*DBP were aligned as described above. The multiple sequence alignment showed that both peptides selected as universal epitopes (1635 and 1638) are conserved among the different analyzed sequences (data not shown).

Homology-based structure prediction model

The position of polymorphic residues obtained by sequence alignment, as well as peptides 1635 and 1638 in the *P. vivax* DBP region II structure prediction model is shown in Fig. 3. According to the results, peptides 1635 and 1638 are located on an opposite region to the one were polymorphic residues are localized.

Discussion

*Pv*DBP is one of the main antigens to be included in the design of a vaccine against *P. vivax* malaria. The main binding domain to the *Pv*DBP is the so-called region II, located in the N-terminal cysteine-rich region (comprising 12 cysteine residues conserved among some *Plasmodium* species) [20] which contains the necessary binding motifs to the DARC receptor [21–23]. In this study, the ten peptides previously reported by Ocampo et al. [10] as having high binding ability to reticulocytes were selected, which are mainly located in *Pv*DBP region II as shown in Fig. 1. Similarly, peptide-binding assays were carried out with HLA-DRB1*0101, HLA-DRB1*0401, HLA-DRB1*0701, HLA-DRB1*1101, and HLA-DRB1*1301 since these molecules are among the 10 most prevalent ones in Afroamerican and Asian populations, where malaria is endemic [24].

The results suggest that peptides 1635 and 1638 could be considered as universal epitopes since they bound to the larger number of HLA-DR molecules. Both interacted better with the HLA-DRB1*0401 molecule since less peptide concentration was needed to displace the HA indicator peptide by 50% (75.19 μM and 64.51 μM, respectively) (Table 2). These results are particularly relevant since DRB1*0401 allele is more prevalent among American (29.8%) and Asian (40.4%) populations compared to other populations [24]. The methodology followed here has been used in previous studies on *P. falciparum* MSP1 [25] for determining the concentration at which a particular peptide interacts with an HLA-DR molecule.

The use of bioinformatics tools for predicting peptides binding to specific MHC molecules has been suggested by Nielsen et al. [26]. Nevertheless, it should be noted that the confidence level for predicting epitopes to MHC class II molecules is not usually very high (approximately 50%) because these molecules accept a wider range of peptides in size and binding registers [16]. SYFPEITHI binding prediction for the HLA-DRB1*0101 molecule (Table 3A) did not agree with the experimental results (Fig. 2A), finding each a single different peptide. On the contrary, the tool's prediction (Table 3B) agreed with the experimental data obtained for peptide 1635 with the HLA-DRB1*0401 molecule (Fig. 2B) but did not predict binding of peptide 1638, which experimentally bound to this same molecule. Regarding HLA-DR7 and HLA-DR11 molecules (data not shown), the program predicted no epitopes, whereas the experimental results showed six high binding epitopes for the HLA-DR7 molecule and three for the HLA-DR11 molecule (Fig. 2C and D). This comparison between experimental and theoretical data sets suggest that class II binding prediction tools are useful, but they are to be used with caution, corroborating their results experimentally, given the inherent complexity of the binding process in MHC II molecules and the wide range of antigenic peptides that they can bind and present to T cells [14,27] for triggering an effective immune response [19].

According to the sequence alignment analysis carried out for peptide 1635, there are no changes in the amino acid positions in the first SYFPEITHI-predicted epitope. For the second one, the shift

in P9 anchor from Glutamine to Arginine apparently does not interfere with the right adjustment of peptide 1635 carrying such variation into the HLA-DR4 molecule's P9 pocket, since both variants have the same prediction score (26); nevertheless, such assumption has to be tested experimentally.

The obtained results were compared to literature reports, confirming that even though *Pv*DBP region II is highly variable [11,28,29], peptides 1635 and 1638 are conserved among the different *P. vivax* isolates. Other studies analyzing different region II allelic variants have found a similar adhesion to RBCs (positive for DARC), which suggest that the polymorphism found in this study has no significant effect on the parasite's binding to host cells [29] and that the variability on *Pv*DBP region II does not interfere with parasite's invasion to reticulocytes. Ranjan and Chitnis [30] reported that the critical binding motifs on DBP region II map to a region between amino acids 258–429, which includes peptide 1635 (amino acids 408–427). However, peptide 1638 does not fall within this region (amino acids 469–488).

Singh et al. [22] in their DBP structural analysis with *Plasmodium knowlesi* (a parasitic species infecting primates) revealed that highly polymorphic regions are located on an opposite surface to the one were the DARC contacting residues are located, which indicates a possible mechanism for evading the human immune system's surveillance while preserving the functional features of this protein. Additionally, the authors suggest that this mechanism can be extrapolated to *P. vivax* and this polymorphic region might be located on an opposite position to the DARC contact residues. Bearing this in mind, the 3D structure of *Pv*DBP region II Sal-I strain (Fig. 3) was here obtained based on the DBP region II structure of *P. knowlesi* (PDB 2C6J), finding that the polymorphic residues localization is opposite to peptides 1635 and 1638, thus agreeing with Singh et al. [22]. The results here presented are also supported by a study from Ocampo et al. [10], showing that *Pv*DBP contains peptides binding with high ability to reticulocytes and that particularly, peptides 1625, 1631, 1635, and 1639 are capable of specifically and significantly inhibiting binding of the radiolabeled-recombinant protein to reticulocytes.

In conclusion, the results presented in this study support the inclusion of peptides 1635 and 1638, considered to be universal epitopes, as promising candidates for developing a multi-antigenic, multi-stage vaccine against *P. vivax* given their high binding ability to reticulocytes, their opposed localization with respect to polymorphic residues and their binding profile to some of the most prevalent HLA-DR molecules among Afroamerican and Asian populations, which are the most vulnerable to malaria.

Acknowledgments

Special gratitude goes to Professor Manuel Elkin Patarroyo for all his valuable comments. This research was supported by the "Instituto Colombiano para el Desarrollo de la Ciencia 'Francisco Jose de Caldas' (COLCIENCIAS)" contract RC-2008. Nora Martinez patiently helped translating the manuscript and Alvaro Mongui helped with the artwork.

References

[1] L.J. Carvalho, C.T. Daniel-Ribeiro, H. Goto, Malaria vaccine: candidate antigens, mechanisms, constraints and prospects, Scand. J. Immunol. 56 (2002) 327–343.
[2] J. Cole-Tobian, C.L. King, Diversity and natural selection in *Plasmodium vivax* Duffy binding protein gene, Mol. Biochem. Parasitol. 127 (2003) 121–132.
[3] B. Sina, Focus on *Plasmodium vivax*, Trends Parasitol. 18 (2002) 287–289.
[4] K. Mendis, B.J. Sina, P. Marchesini, R. Carter, The neglected burden of *Plasmodium vivax* malaria, Am. J. Trop. Med. Hyg. 64 (2001) 97–106.
[5] M.R. Galinski, J.W. Barnwell, *Plasmodium vivax*: merozoites, invasion of reticulocytes and considerations for malaria vaccine development, Parasitol. Today 12 (1996) 20–29.
[6] J.H. Adams, B.K. Sim, S.A. Dolan, X. Fang, D.C. Kaslow, L.H. Miller, A family of erythrocyte binding proteins of malaria parasites, Proc. Natl. Acad. Sci. USA 89 (1992) 7085–7089.
[7] L.H. Miller, S.J. Mason, D.F. Clyde, M.H. McGinniss, The resistance factor to *Plasmodium vivax* in blacks. The Duffy-blood-group genotype, FyFy, N. Engl. J. Med. 295 (1976) 302–304.
[8] C.E. Chitnis, M.J. Blackman, Host cell invasion by malaria parasites, Parasitol. Today 16 (2000) 411–415.
[9] C.E. Chitnis, L.H. Miller, Identification of the erythrocyte binding domains of *Plasmodium vivax* and *Plasmodium knowlesi* proteins involved in erythrocyte invasion, J. Exp. Med. 180 (1994) 497–506.
[10] M. Ocampo, R. Vera, L. Eduardo Rodriguez, H. Curtidor, M. Urquiza, J. Suarez, J. Garcia, A. Puentes, R. Lopez, M. Trujillo, E. Torres, M.E. Patarroyo, *Plasmodium vivax* Duffy binding protein peptides specifically bind to reticulocytes, Peptides 23 (2002) 13–22.
[11] E. Ampudia, M.A. Patarroyo, M.E. Patarroyo, L.A. Murillo, Genetic polymorphism of the Duffy receptor binding domain of *Plasmodium vivax* in Colombian wild isolates, Mol. Biochem. Parasitol. 78 (1996) 269–272.
[12] R.A. Houghten, General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen–antibody interaction at the level of individual amino acids, Proc. Natl. Acad. Sci. USA 82 (1985) 5131–5135.
[13] F. Sinigaglia, P. Romagnoli, M. Guttinger, B. Takacs, J.R. Pink, Selection of T cell epitopes and vaccine engineering, Methods Enzymol. 203 (1991) 370–386.
[14] J.M. Calvo-Calle, J. Hammer, F. Sinigaglia, P. Clavijo, Z.R. Moya-Castro, E.H. Nardin, Binding of malaria T cell epitopes to DR and DQ molecules in vitro correlates with immunogenicity in vivo: identification of a universal T cell epitope in the *Plasmodium falciparum* circumsporozoite protein, J. Immunol. 159 (1997) 1362–1373.
[15] M.N. Liang, C. Beeson, K. Mason, H.M. McConnell, Kinetics of the reactions between the invariant chain (85–99) peptide and proteins of the murine class II MHC, Int. Immunol. 7 (1995) 1397–1404.
[16] H. Rammensee, J. Bachmann, N.P. Emmerich, O.A. Bachor, S. Stevanovic, SYFPEITHI: database for MHC ligands and peptide motifs, Immunogenetics 50 (1999) 213–219.
[17] H.G. Rammensee, T. Friede, S. Stevanoviic, MHC ligands and peptide motifs: first listing, Immunogenetics 41 (1995) 178–228.
[18] C. Parra-Lopez, J.M. Calvo-Calle, T.O. Cameron, L.E. Vargas, L.M. Salazar, M.E. Patarroyo, E. Nardin, L.J. Stern, Major histocompatibility complex and T cell interactions of a universal T cell epitope from *Plasmodium falciparum* circumsporozoite protein, J. Biol. Chem. 281 (2006) 14907–14917.
[19] A.K. Sato, J.A. Zarutskie, M.M. Rushe, A. Lomakin, S.K. Natarajan, S. Sadegh-Nasseri, G.B. Benedek, L.J. Stern, Determinants of the peptide-induced conformational change in the human class II major histocompatibility complex protein HLA-DR1, J. Biol. Chem. 275 (2000) 2165–2173.
[20] S. Dutta, J.R. Daugherty, L.A. Ware, D.E. Lanar, C.F. Ockenhouse, Expression, purification and characterization of a functional region of the *Plasmodium vivax* Duffy binding protein, Mol. Biochem. Parasitol. 109 (2000) 179–184.
[21] D. Hans, P. Pattnaik, A. Bhattacharyya, A.R. Shakri, S.S. Yazdani, M. Sharma, H. Choe, M. Farzan, C.E. Chitnis, Mapping binding residues in the *Plasmodium vivax* domain that binds Duffy antigen during red cell invasion, Mol. Microbiol. 55 (2005) 1423–1434.
[22] S.K. Singh, R. Hora, H. Belrhali, C.E. Chitnis, A. Sharma, Structural basis for Duffy recognition by the malaria parasite Duffy-binding-like domain, Nature 439 (2006) 741–744.
[23] K.M. VanBuskirk, E. Sevova, J.H. Adams, Conserved residues in the *Plasmodium vivax* Duffy-binding protein ligand domain are critical for erythrocyte receptor recognition, Proc. Natl. Acad. Sci. USA 101 (2004) 15754–15759.
[24] S. Southwood, J. Sidney, A. Kondo, M.F. del Guercio, E. Appella, S. Hoffman, R.T. Kubo, R.W. Chesnut, H.M. Grey, A. Sette, Several common HLA-DR types share largely overlapping peptide binding repertoires, J. Immunol. 160 (1998) 3363–3373.
[25] L.E. Vargas, C.A. Parra, L.M. Salazar, F. Guzman, M. Pinto, M.E. Patarroyo, MHC allele-specific binding of a malaria peptide makes it become promiscuous on fitting a glycine residue into pocket 6, Biochem. Biophys. Res. Commun. 307 (2003) 148–156.
[26] M. Nielsen, C. Lundegaard, P. Worning, C.S. Hvid, K. Lamberth, S. Buus, S. Brunak, O. Lund, Improved prediction of MHC class I and class II epitopes using a novel Gibbs sampling approach, Bioinformatics 20 (2004) 1388–1397.
[27] J. Hammer, B. Takacs, F. Sinigaglia, Identification of a motif for HLA-DR1 binding peptides using M13 display libraries, J. Exp. Med. 176 (1992) 1007–1013.
[28] T. Tsuboi, S.H. Kappe, F. al-Yaman, M.D. Prickett, M. Alpers, J.H. Adams, Natural variation within the principal adhesion domain of the *Plasmodium vivax* duffy binding protein, Infect. Immun. 62 (1994) 5581–5586.
[29] J. Xainli, J.H. Adams, C.L. King, The erythrocyte binding motif of *Plasmodium vivax* duffy binding protein is highly polymorphic and functionally conserved in isolates from Papua New Guinea, Mol. Biochem. Parasitol. 111 (2000) 253–260.
[30] A. Ranjan, C.E. Chitnis, Mapping regions containing binding residues within functional domains of *Plasmodium vivax* and *Plasmodium knowlesi* erythrocyte-binding proteins, Proc. Natl. Acad. Sci. USA 96 (1999) 14067–14072.