BRIEF COMMUNICATION

Comparative molecular and three-dimensional analysis of the peptide–MHC II binding region in both human and *Aotus* MHC-DRB molecules confirms their usefulness in antimalarial vaccine development

M. E. Patarroyo · G. Cifuentes · J. Baquero

Abstract A vaccine against malaria is desperately needed, and *Aotus* monkeys are highly susceptible to experimental infection with malarial parasites. A thorough analysis of this monkey's immune system molecules was thus undertaken in our institute. Cloning and sequencing, followed by three-dimensional analysis, has revealed high homology with some HLA-DRB1 molecules in terms of their peptide binding region pockets. Molecules such as HLA-DRB1*03, 11, 08, and HLA-DRB1*04 are so similar to *Aotus* MHC-DRB molecules that peptides identified as binding to these molecules and inducing protective immunity in these monkeys could be used in humans without further refinement, while small modifications seem to be needed for those binding to HLA-DRB1*07, HLA-DRB1*15, 16, and HLA-DRB1*10-like molecules, making this New World monkey an excellent model for tailor-made vaccine development, especially against malaria.

Keywords Malaria · *Aotus* · MHC-DRB · *P. falciparum* · ^{1}H-NMR · MHC II–peptide–TCR complex

Introduction

Understanding the three-dimensional structure of the macromolecular complex constituted by proteins from the major histocompatibility complex (MHC), peptides, and T cell receptors (TCRs) (MHC–peptide–TCR) has led to a better understanding of those molecular processes involved in antigen presentation and developing an appropriate immune response (Garcia et al. 1999; Reinherz et al. 1999; Hennecke et al. 2000).

The genetic region encoding human MHC class II molecules determines the synthesis of three membrane protein isotypes, named HLA-DP, -DQ, and -DR, consisting of a molecular heterodimer having two chains: alpha (α), almost monomorphic encoded by the HLA-DRA region, and beta (β), encoded by the HLA-DRB region having wide genetic polymorphism (Marsh et al. 2000). This HLA-DRB region contains nine genes, five of which are pseudogenes and the other four (HLA-DRB1, 3, 4, and 5) are translated, HLA-DRB1* having the most polymorphism. The molecules encoded by HLA-DRB1* display wide genetic polymorphism in humans, having 16 alleles and ~250 variants able to present peptide antigens to TCR, to conform the MHC II–peptide–TCR complex and induce an optimal immune response.

When developing vaccines (particularly against malaria), clinical studies (especially phase 3 trials) are surrounded by a high degree of uncertainty due to the large number of confounding factors, such as the individual's and the parasite's genetic variabilities; lack of knowledge of the immunological principles determining a protective immune response; the amount of inoculum injected during infected *Anopheles* mosquito's bites; the physical, chemical, and biological characteristics of the antigens used for inducing immune protection; the variability of the vaccine batches being used for immunoprophylaxis; and many more. An appropriate experimental model for solving these problems is the *Aotus* monkey, due to its extreme susceptibility to malaria and other human infectious diseases and the very high similarity between its immune system molecules and those of humans.

M. E. Patarroyo (✉) · G. Cifuentes · J. Baquero
Fundación Instituto de Inmunología de Colombia (FIDIC),
Carrera. 50 No. 26-00 Bogotá, Colombia
e-mail: mepatarr@mail.com

M. E. Patarroyo
Universidad Nacional de Colombia,
Bogotá, Colombia

Previously, an isolation and characterization of the MHC-DRB1 exon 2 from *Aotus nancymaae*, *Aotus nigriceps*, and *Aotus vociferans* shows that MHC-DRB in *Aotus* was divided in 12 allelic lineages (containing 15 sequence groups), which have striking convergence with human lineages: overall mean homology value was greater than 90% and top homology limit exceeded 85% in all comparisons. For the pockets' positions [the most variable residues in peptide binding region (PBR)], the mean value was around 80% and top homology reached 100% (Niño-Vásquez et al. 2000; Suarez et al. 2006).

All 15 *Aotus* MHC-DRB sequence groups were associated with HLA-DRB sequences at the level of residues directly involved in peptide binding (Suarez et al. 2006). The following association with HLA-DRB was found: HLA-DRB1*03 converged with *Aotus* MHC-DRB1*03 GA; HLA-DRB1*1130 converged with *Aotus* MHC-DRB*W44; HLA-DRB1* 08 converged with *Aotus* MHC-DRB1*03 GB; HLA-DRB1*04 and HLA-DRB1*1122 converged with *Aotus* MHC-DRB*W45, W46, and W47; HLA-DRB1*0422 and HLA-DRB3 converged with *Aotus* MHC-DRB*W18 and DRB3*06 GA; HLA-DRB1*07 and HLA-DRB1*09 converged with *Aotus* MHC-DRB*W30 and W38; and HLA-DRB1*15 and DRB1*16 was related to *Aotus* MHC-DRB*W29, -DRBW*42, -DRB6*03 GB, -DRB1*03 GC, and -DRB*W13, and HLA-DRB4 and HLA-DRB1*10 converged with *Aotus* MHC-DRB*W41 and W43 (Suarez et al. 2006).

Molecular knowledge of *Aotus* MHC-DRB1-like proteins could thereby lead to the logical and rational design of synthetic, multiantigen, subunit-based vaccines that could be recognized by both *Aotus* and human HLA-DRB molecules, thus avoiding the risks and costly, prolonged clinical trials involving thousands of human beings to test a single molecule or a group of them, accompanied by all their inherent ethical, scientific, and logistical problems.

Conforming pocket amino acids are shown in Fig. 1a and Tables 1 and 2 and were chosen according to Stern et al. (1994), Ghosh et al. (1995), Hennecke et al. (2000), and Cardenas et al. (2005a,b). The coordinates were extracted from Protein Data Bank (PDB) records for HLA-DRβ1*0301-CLIP (code 1A6A in PDB) (Ghosh et al. 1995) and HLA-DRB1*0401-HA (code 1J8H in PDB) (Hennecke et al. 2000) crystallized complexes according to the amino acids involved in the conformation of pockets 1, 4, 6, and 9 to compare the molecular structures of the different pockets between HLA-DRB1* molecules and their *Aotus* MHC DRB1* homologue molecules. Insight II software program was used (Biopolymers 2000) to build the model.

Pocket 1

The most important pocket in the antigens' anchoring (Cardenas et al. 2005a) displays a genetic dimorphism present in residue β86 in all alleles from both *Aotus* and
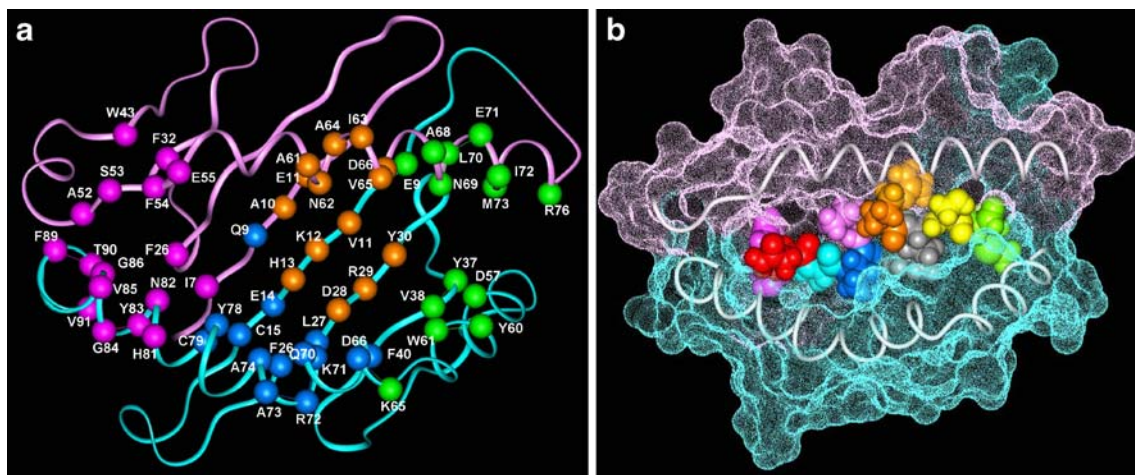

Fig. 1 a Ribbon diagram of the HLA-DRB1*0401 (Dessen et al. 1997) molecule, showing the α chain in *pink* and the β chain in *light blue*. The localizations of residues conforming pocket 1 are shown in *fuchsia*, pocket 4 in *blue*, pocket 6 in *light brown*, and pocket 9 in *green*. **b** Docking of immunogenic, *P. falciparum* malaria protection-inducing peptide 24112 (Patarroyo et al. 2005) in *Aotus* monkeys *Aona* DRB3*0604 (HLA-DRB1*0422-like) allele, which specifically binds to the HLA-DRB1*0401 molecule (Dessen et al. 1997). Van der Waals surface color code for 24112 peptide amino acids and those fitting into class-II molecules pockets (*underlined*): *fuchsia* (pocket 1), *red* (P2), *turquoise* (P3), *blue* (pocket 4), *rose* (P5), *light brown*

(pocket 6), *gray* (P7), *yellow* (P8), and *green* (pocket 9). The docking of these two molecules provided a 2.932 root-mean-square deviation, suggesting that, in spite of marked differences in methodology when obtaining their three-dimensional structures (X-ray crystallography and ^{1}H-NMR), this immunogenetic, protection-inducing peptide in *Aotus* monkeys fits almost perfectly into HLA-DRB1*0401 PBR. These structural findings give strong support to the use of these monkeys in the development of multiantigenic, tailor-made, subunit-based synthetic vaccines (Patarroyo et al. 2005). Note that, besides canonical pockets 1, 4, 6, and 9, residue P7 is also partially buried in the PBR, suggesting the existence of a pocket 7 in this allele

Table 1 Amino acid sequence alignment of residues conforming pockets 1 and 4 in HLA alleles sharing homology with the most frequent *Aona* DRB alleles, according to Suarez et al. (2006)

a

POCKET 1

	81	82	83	84	85	86	89	90	91
HLA-DRB1*0301	H	N	Y	G	V	V	F	T	V
•Aona DRB1*0311	-	-	-	-	-	-	-	-	-
•Aona DRB1*0312	-	-	-	-	-	-	-	-	-
•Aona DRB1*0304	-	-	-	-	-	G	-	-	-
HLA DRB1*1130	H	N	Y	G	V	G	F	T	V
Aona DRB*W4401	-	-	-	-	-	-	-	-	-
HLA-DRB1*0801	H	N	Y	G	V	G	F	T	V
•Aona DRB1*0302	Y	-	-	-	-	A	-	-	-
Aona DRB1*0321	Y	-	-	-	-	A	-	-	-
HLA-DRB1*0403	H	N	Y	G	V	V	F	T	V
Aona DRB*W4701	-	-	-	-	-	-	-	-	-
Aona DRB*W470401	-	-	-	-	-	G	-	-	-
HLA-DRB1*0422	H	N	Y	G	V	V	F	T	V
•Aona DRB3*0603	-	-	-	-	-	-	-	-	-
Aona DRB*W1803	-	-	-	-	-	-	-	-	-
•Aona DRB3*0604	-	-	-	-	-	G	-	-	-
•Aona DRB*W1801	-	-	-	-	-	G	-	-	-
•Aona DRB3*0602	-	-	-	-	-	F	-	-	-
HLA-DRB1*0701	H	N	Y	G	V	G	F	T	V
Aona DRB*W3801	-	-	-	-	-	-	-	-	-
Aoni DRB*W3801	-	-	-	-	-	V	-	-	-
HLA-DRB1*090102	-	-	-	-	-	-	-	-	-
HLA DRB1*150101	H	N	Y	G	V	V	F	T	V
Aoni DRB1*0305	-	-	-	-	-	-	-	-	-
Aona DRB1*0327	-	-	-	-	-	G	-	-	-
Aona DRB*W2906	-	-	-	-	-	-	-	-	-
Aona DRB*W2907	-	-	-	-	-	-	-	A	-
•Aona DRB*W1302	-	-	-	-	-	-	-	-	-
Aona DRB*W1308	-	-	-	-	-	A	-	-	-
Aoni DRB*W1307	-	-	-	-	-	G	-	-	-
Aovo DRB*W1304	-	-	-	-	-	-	-	-	A
•Aona DRB*W2901	-	-	-	-	-	F	-	-	-
HLA DRB1*160101	-	-	-	-	-	G	-	-	-
HLA-DRB1*1001	H	N	Y	G	V	G	F	T	V
Aoni DRB*W4301	-	-	-	-	-	-	-	-	-
Aoni DRB*W4304	-	-	-	-	-	V	-	-	-
Aoni DRB*W4303	-	-	-	-	-	-	L	-	-
HLA DRB4*0101	Y	-	-	-	-	-	-	-	-

POCKET 4

	14	15	.	26	27	.	70	71	72	73	74	.	78	79
HLA-DRB1*0301	E	C	-	Y	L	-	Q	K	R	G	R	-	Y	C
Aona DRB1*031701	-	-	-	·	·	-	·	·	·	·	·	-	·	·
•Aona DRB1*0304	-	-	-	F	·	-	·	·	·	·	·	-	·	·
Aona DRB1*0319	-	-	-	H	·	-	·	·	·	·	·	-	·	·
•Aona DRB1*0303	-	-	-	·	·	-	·	·	·	·	Q	-	·	·
•Aona DRB1*0307	-	-	-	·	·	-	R	·	·	·	·	-	·	·
Aovo DRB1*0301	-	-	-	·	·	-	R	·	·	·	Q	-	·	·
HLA-DRB1*1130	E	C	-	F	L	-	D	R	R	A	A	-	Y	C
Aona DRB*W4401	-	-	-	Y	·	-	·	H	·	·	·	-	·	·
HLA-DRB1*0801	E	C	-	F	L	-	D	R	R	A	L	-	Y	C
•Aona DRB1*0302	-	-	-	·	·	-	E	·	·	·	·	-	·	·
•Aona DRB1*0301	-	-	-	Y	·	-	E	·	·	·	·	-	·	·
Aona DRB1*0326	-	-	-	·	·	-	E	·	·	·	·	-	·	·
HLA-DRB1*0422	E	C	-	F	L	-	Q	K	R	G	R	-	Y	C
•Aona DRB3*0605	-	-	-	·	·	-	·	·	·	·	·	-	·	·
•Aona DRB3*0601	-	-	-	Y	·	-	·	·	·	·	·	-	·	·
Aona DRB3*0620	V	-	-	·	·	-	·	·	·	·	·	-	·	·
Aoni DRB*W1801	-	-	-	·	·	-	L	·	·	·	·	-	·	·
•Aona DRB*W2902	-	-	-	L	·	-	L	·	·	·	·	-	·	·
•Aona DRB*W2903	-	-	-	L	·	-	L	·	·	·	·	-	·	·
Aona DRB3*0617	-	-	-	Y	·	-	·	·	·	·	Q	-	·	·
Aona DRB3*0618	-	-	-	Y	·	-	·	·	·	·	Q	-	·	·
HLA-DRB3*0201	-	-	-	·	·	-	·	·	·	·	Q	-	·	·
HLA-DRB1*0403	E	C	-	F	L	-	Q	R	R	A	E	-	Y	C
Aona DRB*W4703	-	-	-	·	·	-	D	·	·	·	Q	-	·	·
Aona DRB*W4709	-	-	-	·	·	-	D	·	·	·	Q	-	·	·
Aona DRB*W4711	-	-	-	·	·	-	E	·	·	·	·	-	·	·
Aona DRB*W4601	-	-	-	·	·	-	D	·	·	·	Q	-	·	·
HLA DRB1*1122	-	-	-	·	·	-	D	·	·	·	A	-	·	·
HLA-DRβ1*0701	K	C	-	F	L	-	D	R	R	G	Q	-	V	C
Aona DRB*W3801	E	-	-	·	·	-	·	·	·	·	A	-	·	·
Aona DRB*W3802	E	-	-	·	·	-	V	·	·	·	A	-	·	·
•Aona DRB*W3001	E	-	-	Y	·	-	·	·	·	·	A	S	-	Y	·
HLA-DRB1*090102	E	-	-	Y	·	-	R	·	·	·	A	E	-	·	·
HLA DRB1*150101	E	C	-	F	L	-	Q	A	R	A	A	-	Y	C
Aona DRB*W1303	-	-	-	·	·	-	E	T	·	·	·	-	·	·
Aona DRB*W1311	-	-	-	·	·	-	E	T	·	·	·	-	·	·
HLA DRB1*160101	-	-	-	·	·	-	D	R	·	·	·	-	·	·
HLA DRB1*150101	E	C	-	F	L	-	Q	A	R	A	A	-	Y	C
Aoni DRB1*0305	-	-	-	Y	·	-	E	T	·	·	·	-	·	·
•Aona DRB1*0313	-	-	-	·	·	-	E	T	·	·	·	-	·	·
•Aona DRB1*0314	-	-	-	Y	·	-	E	T	·	·	·	-	·	·
Aona DRB1*0327	-	-	-	Y	·	-	D	R	·	·	Q	-	·	·
HLA DRB1*160101	-	-	-	·	·	-	D	R	·	·	·	-	·	·
HLA DRB1*150101	E	C	-	F	L	-	Q	A	R	A	A	-	Y	C
•Aona DRB*W2901	-	-	-	L	·	-	Y	L	·	·	·	-	·	·
Aoni DRB*W2903	-	-	-	L	·	-	Y	L	·	·	·	-	C	·
•Aona DRB3*0608	-	-	-	Y	·	-	Y	L	·	·	·	-	·	·
•Aona DRB1*0316	-	-	-	Y	·	-	Y	L	·	·	·	-	·	·
Aona DRB*W4201	-	-	-	F	·	-	Y	L	·	·	·	-	·	·
HLA DRB1*160101	-	-	-	·	·	-	D	R	·	·	·	-	·	·
HLA-DRB1*1001	E	C	-	L	L	-	Q	R	R	A	A	-	Y	C
Aoni DRB*W4301	-	-	-	·	·	-	N	·	·	·	·	-	·	·
Aoni DRB*W4302	-	-	-	·	·	-	N	·	·	·	·	-	·	·
HLA DRB4*0101	-	-	-	N	·	-	R	·	·	·	E	-	·	·

humans (Demotz et al. 1993), where (due to the hydrophobic nature of the amino acids comprising it) variation β86G allows the fitting of voluminous apolar amino acids such as W (227 Å^3), Y (194 Å^3), and F (190 Å^3) (Fig. 2b). The presence of the other dimorphic amino acid (β86V) allows the fitting of smaller-sized apolar residues, such as L and I (167 Å^3), M (163 Å^3), or V (140 Å^3), due to the steric hindrance imposed on this pocket by β86V side-chains, clearly seen in the stereo view shown in Fig. 2a,b.

Aotus MHC-DRB presents variation β86A in few human alleles but is predominant in *Aotus* MHC-DRB1* 0301, 0302, 0309, and 0320 to 0326 (Table 1) (members of the HLA-DRB1* 08-like group), allowing the fitting of Y and F, but less likely that of W, due to this amino acid's larger volume. Variation β86F occurs less frequently in *Aotus* (~15%) in some individuals presenting *Aotus* MHC-DRB*W0602 (HLA-DRB1*0422-like) and *Aotus* MHC-DRB*W2901 (HLA-DRB1*15-like HLA-DRB1*16-like) (Table 1), allowing the fitting of small apolar residues, such as T (116 Å^3), A (89 Å^3), P (113 Å^3), or even G (60 Å^3). Variations β86G, β86V, and β86A were thus identical to those described for

Table 2 Amino acid sequence alignment of residues conforming pockets 6 and 9 in HLA alleles sharing homology with the most common *Aona* DRB alleles, according to Suarez et al. (2006)

b   **POCKET 6**

	11	12	13	.	28	29	30	.	40	.	66
HLA-DRB1*0301	S	T	S	-	D	R	Y	-	F	-	D
•Aona DRB1*0305	T	-	-	-	-	-	-	-	-	-	-
•Aona DRB1*0307	T	-	-	-	-	-	-	-	-	-	-
Aona DRB1*031701	T	-	-	-	-	-	-	-	-	-	-
HLA DRB1*1130	L	K	S	-	D	R	Y	-	F	-	D
Aona DRB*W4401	A	-	-	-	-	-	-	-	-	-	-
HLA-DRB1*0801	S	T	G	-	D	R	Y	-	F	-	D
•Aona DRB1*0301	T	-	S	-	-	-	-	-	-	-	-
•Aona DRB1*0308	T	-	S	-	-	-	-	-	-	-	-
HLA-DRB1*0403	V	K	H	-	D	R	Y	-	F	-	D
Aona DRB*W4701	-	-	-	-	-	-	-	-	-	-	-
Aona DRB*W4708	-	-	-	-	-	-	-	-	-	-	-
Aona DRB*W4709	-	-	D	-	-	-	-	-	-	-	-
Aona DRB*W4711	-	-	-	-	-	-	-	-	-	-	-
Aona DRB*W4501	-	-	-	-	-	-	-	-	-	-	-
Aona DRB*W4601	F	-	P	-	-	-	-	-	-	-	-
HLA DRB1*1122	-	-	-	-	-	-	-	-	-	-	-
HLA-DRB1*0422	V	K	H	-	D	R	Y	-	F	-	D
Aona DRB3*0620	-	-	-	-	-	-	-	-	-	-	-
Aona DRB3*0622	A	-	-	-	-	-	-	-	-	-	-
•Aona DRB3*0601	-	-	-	-	-	-	-	-	-	-	-
HLA-DRB1*0701	G	K	Y	-	E	R	L	-	F	-	D
Aona DRB*W3801	A	-	-	-	-	-	H	-	-	-	-
•Aona DRB*W3001	V	-	-	-	-	-	-	-	-	-	-
HLA-DRB1*090102	D	-	F	-	H	-	G	-	-	-	-
HLA DRB1*150101	P	K	R	-	D	R	Y	-	F	-	D
•Aona DRB*W1302	F	-	P	-	-	-	-	-	-	-	-
Aona DRB*W1303	F	-	L	-	-	-	-	-	-	-	-
HLA DRB1*160101	-	-	-	-	-	-	-	-	-	-	-
HLA DRB1*150101	P	K	R	-	D	R	Y	-	F	-	D
Aoni DRB1*0305	T	-	S	-	-	-	-	-	-	-	-
HLA DRB1*160101	-	-	-	-	-	-	-	-	-	-	-
HLA DRB1*150101	P	K	R	-	D	R	Y	-	F	-	D
•Aona DRB3*0608	V	-	H	-	-	-	-	-	-	-	E
•Aona DRB1*0316	T	T	S	-	-	-	-	-	-	-	E
•Aona DRB*W2901	T	-	S	-	Q	-	-	-	-	-	E
Aoni DRB*W4201	V	-	D	-	E	-	-	-	-	-	E
HLA DRB1*160101	-	-	-	-	-	-	-	-	-	-	-
HLA-DRB1*1001	V	K	F	-	E	R	R	-	Y	-	D
Aoni DRB*W4301	-	-	C	-	D	-	H	-	F	-	-
Aona DRB*W4101	-	-	C	-	D	-	H	-	F	-	-
Aoni DRB*W4302	-	-	C	-	D	-	H	-	S	-	-
HLA DRB4*0101	A	-	C	-	I	-	Y	-	-	-	-

POCK ET 9

	9	-	30	-	37	38	-	57	-	60	61	-	65
HLA-DRB1*0301	E	-	Y	-	N	V	-	D	-	Y	W	-	K
•Aona DRB1*0312	F	-	-	-	Y	-	-	-	-	-	-	-	-
Aona DRB1*031701	V	-	-	-	Y	-	-	-	-	-	-	-	-
HLA DRB1*1130	E	-	Y	-	Y	V	-	D	-	Y	W	-	K
Aona DRB*W4401	-	-	-	-	-	-	-	T	-	-	-	-	-
HLA-DRB1*0801	E	-	Y	-	Y	V	-	S	-	Y	W	-	K
•Aona DRB1*0301	F	-	-	-	-	-	-	-	-	-	-	-	-
Aona DRB1*0321	F	-	-	-	-	-	-	-	-	-	-	-	-
HLA-DRB1*0403	E	-	Y	-	Y	V	-	D	-	Y	W	-	K
Aona DRB*W4701	-	-	-	-	-	-	-	-	-	-	-	-	-
Aona DRB*W4705	-	-	-	-	-	-	-	-	-	H	-	-	-
Aona DRB*W4708	K	-	-	-	-	-	-	-	-	-	-	-	-
Aoni DRB*W4701	-	-	-	-	-	A	-	-	-	-	-	-	-
Aona DRB*W4501	-	-	-	-	V	-	-	-	-	-	-	-	-
HLA DRB1*1122	-	-	-	-	-	-	-	-	-	-	-	-	-
HLA-DRB1*0422	E	-	Y	-	Y	V	-	D	-	Y	W	-	K
•Aona DRB3*0602	-	-	-	-	-	-	-	-	-	-	-	-	-
•Aona DRB3*0601	-	-	-	-	-	V	-	-	-	-	-	-	-
•Aona DRB3*0607	-	-	-	-	-	S	-	-	-	-	-	-	-
•Aona DRB*W2902	-	-	-	-	-	-	-	-	-	-	-	-	-
•Aona DRB*W1801	-	-	-	-	-	-	-	-	-	-	-	-	Q
Aona DRB*W1804	-	-	-	-	L	-	-	-	-	-	-	-	Q
Aona DRB*W1803	-	-	-	-	F	-	-	-	-	-	-	-	Q
HLA-DRB3*0201	-	-	H	-	-	A	-	-	-	-	-	-	-
HLA-DRB1*0701	W	-	L	-	F	V	-	V	-	S	W	-	K
•Aona DRB*W3001	E	-	-	-	-	-	-	S	-	K	L	-	-
Aona DRB*W3801	E	-	H	-	Y	A	-	T	-	Y	-	-	-
HLA-DRB1*090102	K	-	G	-	-	-	-	-	-	-	-	-	-
HLA DRB1*150101	W	-	Y	-	S	V	-	D	-	Y	W	-	K
Aoni DRB*W2903	E	-	-	-	Y	-	-	-	-	-	-	-	-
Aoni DRB*W1306	E	-	-	-	Y	-	-	-	-	-	-	-	-
•Aona DRB*W2901	E	-	-	-	Y	A	-	-	-	-	-	-	-
•Aona DRB*W1302	E	-	-	-	Y	-	-	-	-	-	F	-	-
Aona DRB*W1303	E	-	-	-	Y	-	-	E	-	-	F	-	-
Aona DRB*W1312	E	-	-	-	F	-	-	T	-	-	F	-	-
Aona DRB*W1307	E	-	-	-	Y	-	-	-	-	-	L	-	-
HLA DRB1*160101	-	-	-	-	-	-	-	-	-	-	-	-	-
HLA DRB1*150101	W	-	Y	-	S	V	-	D	-	Y	W	-	K
Aoni DRB1*0305	F	-	-	-	Y	-	-	-	-	-	F	-	-
•Aona DRB1*0313	F	-	-	-	Y	-	-	E	-	-	F	-	-
Aona DRB1*0327	F	-	-	-	Y	-	-	-	-	-	-	-	-
HLA DRB1*160101	-	-	-	-	-	-	-	-	-	-	-	-	-
HLA-DRB1*1001	E	-	R	-	Y	A	-	D	-	Y	W	-	K
Aona DRB*W4101	K	-	H	-	-	-	-	-	-	-	F	-	-
Aoni DRB*W4301	K	-	H	-	-	-	-	T	-	-	-	-	-
HLA DRB4*0101	-	-	Y	-	-	-	-	-	-	-	-	-	-

humans. Therefore, this pocket, determining the strongest electrostatic PBR binding energy (Cardenas et al. 2005a,b), displays the same binding characteristics in both humans and *Aotus*.

Pocket 4

The almost complete homology of *Aotus* MHC-DRB1*0301 to 0319 and 03170 allelic families with human HLA-DRB1*0301 (Suarez et al. 2006) (Table 1 and Fig. 2a) can be seen in pocket 4. Residues β70Q, β71K, and β74R are the main determinants of the specificity for the fitting of those peptide residues interacting with this pocket in this allele. As β71K (168.6 Å^3) and β74R (173.4 Å^3) are so voluminous in this allele (HLA-DRB1*03-like), the size of this pocket is small, and as they have positive charges, as seen in Fig. 2a, they only allow the fitting of small, negatively charged amino acids,

such as aspartic acid, D (111 Å^3), or small, related noncharged, polar amino acids, such as asparagines, N (114 Å^3), in this pocket (Rammensee et al. 1995).

The steric similarity of changes Rβ70Q and Qβ74R found in a few monkeys carrying the HLA-DRB1*03-like allele suggests that such balanced changes in terms of charge and volume could not have a greater effect on this pocket's size in this allele and, therefore, the corresponding amino acid binding (Fig. 2a).

As variation Yβ26F was found outside pocket 4 in *Aona* DRB*W4401, this variation perhaps does not have a greater impact on this pocket's affinity in *Aotus* HLA-DRB1*1120-like alleles. Something similar could be happening with the Hβ71R variation; this allele's affinity could thus be very similar between *Aotus* and humans in this pocket.

Although pocket 4 has not been described so far in humans, nor its corresponding binding motifs, but, rather, pocket 5 in HLA-DRB1*08, the only difference in *Aotus*
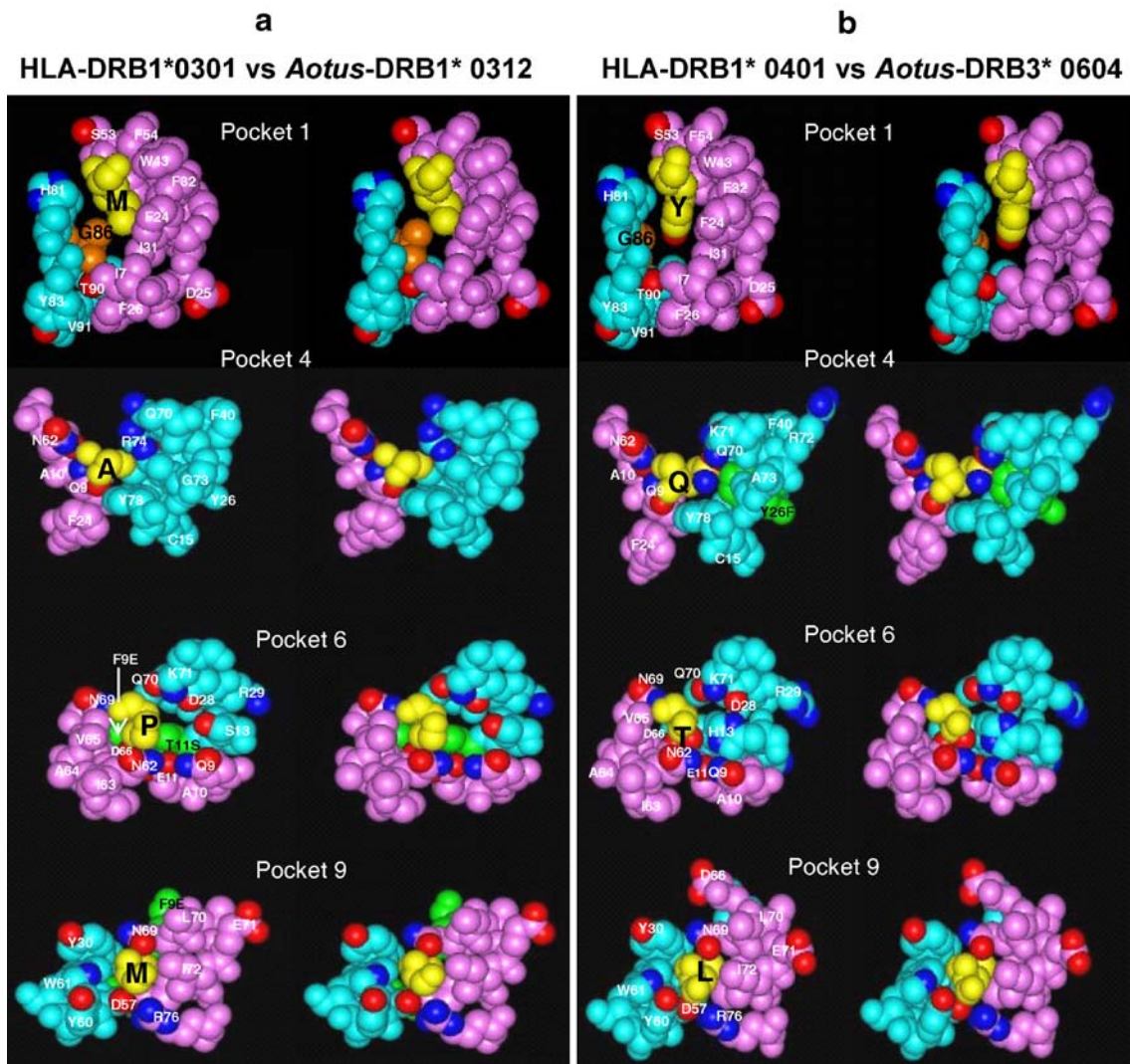
Fig. 2 a α chain residues are in *pink* while β chain ones are in *pale blue*. Stereo view of human HLA-DRB1*0301 (Ghosh et al. 1995) pockets 1, 4, 6, and 9 conforming amino acids compared to *Aotus* MHC-DRB1*0312 (Suarez et al. 2006) highlighting the difference Tβ11S in pocket 6 (in green) without any impact in size and volume, due to the small differences between these two amino acids, and Fβ9E in pocket 9 (in green), having no impact on this pocket due to its lateral localization. **b** Stereo view of human HLA-DRB1*0401 (Dessen et al. 1997) showing amino acid Yβ26F difference with *Aotus* MHC-DRB3*0604 in pocket 4, *highlighted in green*. Note also

in this stereo view the difference in volume of the antigen amino acid (in *yellow*), due to the Vβ86G dimorphism (*light brown*) present in both species in pocket 1. Steric impediment of β86V (*left-hand panel*) allows smaller apolar amino acids such as M, L, I, and V to fit into this pocket (M presented in *yellow*), while Vβ86G (*right-hand panel*), due to its larger space, allows bigger apolar amino acids to fit into this pocket, such as W, F, and Y (*yellow*). The purpose of these stereo-views is to remark on the importance of localizing three-dimensionally genetic differences between these two species in the PBR to determine their impact on peptide binding

MHC-DRB1*03 GB alleles (HLA-DRB1*08) lay in Eβ70D (similar charge but 15 Å³ larger volume for E) suggesting that this pocket in *Aotus* could be a little smaller than in humans (Rammensee et al. 1995).

Regarding alleles *Aotus* MHC-DRB3*0601 to 0607 and 0612 to 0624, the majority of these monkeys showed almost complete identity with HLA-DRB1*0422 (Suarez et al. 2006), but few *Aotus* present variation Qβ74R, which also allows fitting slightly more voluminous, negatively charged amino acids (E), as happens in humans receiving both D and E in this allele. Variation Yβ26F and Lβ26F

localized in the floor of this pocket (Fig. 2b, in green) and Lβ70Q could determine that antigenic, polar, noncharged amino acids, such as N, or apolar ones, such as M, L, I, V, T, S, and A, are accepted better into this pocket. The other residues forming pocket 4 were identical for both *Aotus* and humans and, therefore, could have the same amino acid preferences as in humans for this allele.

The only consistent differences in *Aotus* MHC-DRB*W4702, 4703, 4709, 4711, and 4601 regarding human HLA-DRB1*0403-like were found in Dβ70Q and Qβ74E, which were charge-balanced differences. However,

apolar residues such as L, I, M, V, and A could be allowed to fit (due to the difference of 27 Å^3 in volume between these residues), as they also belong to motifs associated with this allele in humans (Rammensee et al. 1995).

Changes Eβ14K and Aβ73G for allele *Aotus* MHC-DRB*W38, W30 (HLA-DRB1*07-like) could determine the preference for apolar amino acids, such as M, V, T, and S, and some other polar noncharged ones, such as N and Q, and positively charged ones, such as H and K, as happens in humans for this allele; however, the size could be slightly smaller as a consequence of the Aβ73G; the amino acids fitting into this pocket will thereby be quite similar in both *Aotus* and humans (Rammensee et al. 1995).

Pocket 4 in *Aotus* MHC-DRB*W4301and 4302 (similar to HLA-DRB1*1001) having Nβ70Q variations could be a little bit more spacious in *Aotus* MHC-DRB due to the smaller volume of Nβ70Q in *Aotus*; however, because both residues have the same polarity in *Aotus* as in humans MHC-DRB, this primate could prefer more voluminous apolar amino acids, such as L, I, V, and M, or noncharged polar ones, such as Q and N, similar to those found to bind in humans to this pocket (Demotz et al. 1993; Rammensee et al. 1995) in HLA-DRB1*0101, an allele homologous to this in *Aotus* as well as humans. Both molecules are therefore very similar for this pocket in this allele.

Comparing HLA-DRB1*15, 16 alleles (belonging to the HLA-DR51 haplotype) and *Aotus* typed as being DRB*W1301 to DRB*W1313 and DRB*0305, 0314, 0315, and 0327 alleles carrying the Eβ70Q or Dβ70Q and Tβ71A reveals similar volume but a difference in charge in *Aotus* alleles facilitating their accepting large polar residues such as Y, as happen in humans. But differences Yβ70Q and L71A were definitely more marked from the volumetric and electrostatic point of view between *Aotus* MHC-DRB*W2901, DRB3*0608, DRB3*0316, and human HLA-DRB1*15, 16 alleles, making it difficult to compare such allelic variants between humans and *Aotus* monkeys.

Pocket 6

This pocket, greatly determined by the α-chain (pink) (Fig. 1a), contains one of the most interesting electrostatic interactions, as αE11 and αD66, localized in the floor of this pocket, spatially confront each other. These amino acids' carboxylic acids are protonated and stabilized by a network of H-bonds stabilized by three to four water molecules (Fremont et al. 1996). When a negatively charged peptide amino acid, such as glutamic acid (E), is introduced into pocket 6, it enters this network already protonated (Fremont et al. 1996). If a negatively charged amino acid peptide, such as E, is replaced by another

negatively charged one, such as D, which is shorter in a methylene (CH2) group, then shortening this amino acid's lateral chain makes the peptide's P5 residue become conformationally modified (being found to be ~1 Å distant). However, as a consequence of this displacement, a striking rotation is fundamentally induced in residue P8, which now is 10 Å distant from pocket 6. Such rotation induces a weak interaction with the TCR, reducing a peptide's immunogenic activity more than 1,000-fold, making this altered peptide ligand become an antagonic peptide (Kersh et al. 2001) with tremendous consequences in the immune response.

As seen in the stereo view of Fig. 2a, pocket 6 in HLA-DRB1*0301 is a large and spacious, horizontally oriented pocket that, due to its negative charges, can accommodate large, positively charged residues like R (173 Å^3) and K (168.6 Å^3), noncharged polar ones like Q (143 Å^3) and N (114 Å^3), or a negatively charged one such as E (138 Å^3).

Variations Tβ11S (green) in *Aotus* MHC-DRB1*0301 to 0319 and 03170 (HLA-DRB1*03), as well as Yβ32H, seem to have no major impact on this pocket's preference because these variations are found on its lateral face, without influencing either its depth or width (Fig. 2a). For pocket 6, the only Aβ11L change will allow the preferential fitting of large polar residues like R and K as in humans because the other amino acids are identical when compared with HLA-DRB1*11. There are also small volumetric variations in Tβ11S (27.1 Å^3) or Sβ13G (29 Å^3) in DRB1*0302 alleles (HLA-DRB1*08), possibly reducing this pocket's size in *Aotus*, allowing the fitting of large positively charged polar amino acids, such as K, H, and R, as happens in humans. It should be remembered that HLA-DRB1*08 has pocket 5, but no pocket 6.

Some human alleles, such as HLA-DRB1*03, HLA-DRB1*0405, and HLA-DRB1*0407, interact with negatively charged amino acids (E, D) in this pocket. However, this could happen most often in *Aotus* MHC-DRB1*0301 to 0319 and 03170 GA (HLA-DRB1*0301-like) and *Aotus* MHC-DRB*W4701 to 4711 (HLA-DRB1*0403-like) DRB3*0601 to 0624 (HLA-DRB1*0422-like), the most frequent alleles in *Aotus*.

As seen in the stereo view (Fig. 2a,b), this pocket is smaller in HLA-DRB1*0401 due to the larger volume of β13H (153 Å^3) occupied in this pocket when compared to β13S or T (89 and 116 Å^3, respectively) present in HLA-DRB1*0301. Therefore, HLA-DRB1*04-related alleles, with few exceptions (i.e., those carrying Dβ13H and Pβ13H variations), accept noncharged polar amino acids such as Q or N, or apolar ones such as S or T (Rammensee et al. 1995), in humans as well as in *Aotus*, while HLA-DRB1*03, 11, and 08-like *Aona* allelic variations can accept large polar positively charged residues like K and R, as happens in the human situation.

Variation Lβ30H could induce a pH-dependent preference in *Aotus* in this pocket in allele *Aotus* MHC-DRB*W3801 (HLA-DRB1*07-like), observed in some *Aotus* for apolar amino acids, such as S and T, and neutral polar ones, such as N or Q, due to the lost of histidine's pH-dependent protonation characteristics, but these amino acids are also preferred by this human allele. Binding motifs for this pocket in HLA-DRB1*1501 and 1601 have only been recognized so far for pocket 7 in humans (Rammensee et al. 1995).

Interactions with small apolar amino acids (A, G, S, T, and P) could occur with *Aotus* MHC-DRB*W4301 to 4305 and W4101 (and HLA-DRB*1001-like), these being characteristic for this allele (Rammensee et al. 1995); however, Vβ11L (26.6 Å³, smaller), Cβ13F, and Hβ30C (balanced) differences occur in *Aotus*, which could, perhaps, also allow the fitting of slightly larger apolar amino acids, such as V in *Aotus*. This allele belongs to the HLA-DR1 haplotype where the previously mentioned amino acids are preferentially accepted in this pocket. It is worth to remember that special peptide-binding motifs have not been identified for HLA-DRB1*1001, but for HLA-DRB*0101-related alleles.

Homology between *Aotus* alleles *Aotus* MHC-DRB*W47, 46, 45; DRB3*06 GA with HLA-DRB1*04-like; *Aotus* MHC-DRB1*03 GA with HLA-DRB1*03-like; *Aotus* MHC-DRB1*03 GB with HLA-DRB1*08; and DRB*W4401 with HLA DRB1*1130 is therefore complete for this pocket and almost complete for *Aotus* MHC-DRB*W38, 30 with HLA-DRB1*07 and DRB*W43, 41 with HLA-DRB1*1001-like. Variations such as Kβ71E, Kβ71N, Sβ13Y, Sβ11F, and Gα20E have been found in pockets 4 and 6; they dramatically affect these structures and are associated with high levels of CLIP in cells (Doebele et al. 2003). Most of these variations increase stability and reduce CLIP dissociation, but such variations have not been found in the *Aotus* population analyzed here.

Pocket 9

β57D⁺ cf. β57D⁻ (which could be β57S, V, or A in humans) genetic dimorphism found in pocket 9 seems to be the determining factor in this pocket's specificity. When β57S is found instead of β57D in pocket 9, as happens in murine I-Aᵍ⁷ (Corper et al. 2000), or in human HLA-DQ8 (Lee et al. 2001), the salt bridge formed by β57D and α76R is not formed and pocket 9 has a different peptide-groove conformation with an oxyanionic hole in which small antigenic amino acids, having free oxydryl groups in their side-chains, such as D, E, and sometimes S and T, might fit, which in turn could interact with protonated NH2 groups of the α76R, stabilizing the binding of these residues in this pocket. Variation Dβ57E was also found in this pocket in some

Aotus typed as DRB1*0313 (HLA-DRB1*15,16-like), which allows the formation of a salt bridge with α76R due to its polarity, this being similar to that formed by β57D and α76R and accepting the same amino acids as humans.

Genetic variants Tβ57V or Sβ57V, also found in humans, were found in alleles *Aotus* MHC-DRB*W3801, W3001 (HLA-DRB1*07-like), and Tβ57D, as in DRB*W4301 (HLA-DRB1*10-like) and DRB*4401 (HLA-DRB1*1130-like). It could be supposed that this variation leads to the preferential fitting of the apolar amino acids, such as L, I, A, V, S, and P, as it also happens in humans for these alleles.

Amino acid β9W also forms pocket 9 in some human alleles; being its equivalent in *Aotus* β9F. This Fβ9W genetic variation could determine why this pocket preferentially receives voluminous apolar amino acids (Y, F, L, V, and I) in both species, being slightly more spacious in *Aotus* due to the volumetric difference (37.6 Å³) between these two residues. Allele variation Fβ9E (in Fig. 2a), which happens in *Aotus* in alleles DRB1*0301 to 0319 and 03170 (HLA-DRB1*03-like), DRB1*0320 to 0326 (HLA-DRB1*08), could lead to voluminous apolar or aromatic amino acids (Y, L, and F) being accepted, while variation Eβ9W (as in DRB*W3801, W3001 (HLA-DRB1*0701-like), and DRB*W29 and W42 DRB3*06 and DRB*W1301 to W1313 present in most HLA-DRB1*15- and 16-like alleles could allow apolar or positively charged amino acids such as K or R to be received, as happens in the murine system in H-2 I-Eᵏ, I-Eᵈ, I-E³, and I-Eᵇ, these residues being found toward the pocket's rear face. Polymorphic residue β37 is also found in the rear face of pocket 9; however, no specific role has been described for its variations S, N, Y, or V in either humans or *Aotus*. Variations in β60, as happen in *Aotus* MHC-DRB*W38, 30 (HLA-DRβ1*0701-like), seem to have no impact at all because they are outside pocket 9.

Genetic variations in *Aotus* class II molecule pockets, their spatial localization in the PBR, and their physical, chemical and biological characteristics (important for binding to antigen) must thus be known to allow optimum epitope binding to these molecules and, therefore, presentation of the antigen to TCR to induce an appropriate protective immune response. This knowledge will permit a logical and rational methodology for multicomponent, subunit-based, synthetic vaccine development. Based on data provided by *Plasmodium falciparum* merozoite transcriptome analysis (where it was concluded that ~50 proteins were found to be involved in merozoite invasion of RBC), it can be suggested that at least an equal number of peptides for each parasite development stage (of sporozoites and merozoites invading hepatic cells and RBC, respectively) are needed for developing a fully effective antimalarial vaccine.

Our molecular analysis suggests that immunogenic peptides can be developed in *Aotus*, which induce protection against *P. falciparum* malaria, binding with high affinity to human HLA-DRB1*03 and HLA-DRB1*11 (belonging to the HLA-DR52 haplotype) and the related HLA-DRB1*08, alleles (of the HLA-DR8 haplotype) to be used without any further modification for immunizing individuals having these genetic characteristics. Peptides could also be designed which would bind to HLA-DRB1*04 and to HLA-DRB1*07, 09 (which belong to the HLA-DR53 haplotype) for human use, without modifications in the former but with slight changes in the latter. Concerning the latter, the same would be true for peptides binding to some members of the HLA-DRB1*15, 16 (members of the HLA-DR51 haplotype) and HLA-DRB1*10 (member of the HLA-DR1 haplotype), for which slight changes in the volume and charge of residues fitting into pocket 6 could be needed. These data clearly suggest that, knowing these rules, properly modified peptides identified in *Aotus* monkeys, previously typed in their class II molecules by fast methodology such as the reference strand conformational analysis (Baquero et al. 2006), can be used immediately to immunize humans and make a most positive contribution toward human vaccine development (Patarroyo et al. 2004, 2005), rather than immunizing humans in the uncertain, painstaking, long, expensive, and ethically questionable methodologies used up to date.

Analysis by cloning and sequencing nucleotides from TCR α (Favre et al. 1998), TCR β (Moncada et al. 2005), and class I (Cardenas et al. 2005c) or class II MHC or *Aotus* DR (Niño-Vasquez et al. 2000; Suarez et al. 2006), DP (Diaz et al. 2002), DQ (Diaz et al. 2000), CD1 (Castillo et al. 2004), and many more immune system proteins has shown very high homology with those of humans, suggesting that this monkey could be the most appropriate experimental model for studying vaccines, particularly against human malaria caused by *P. falciparum* (Rodriguez et al. 1990) or *Plasmodium vivax* (Pico de Coaña et al. 2003), where it has been shown this *Aotus* monkey it is extremely susceptible.

The above data make this New World monkey an ideal model for developing and obtaining multicomponent, subunit-based, tailor-made synthetic vaccines for those alleles present in >80% of the world's population, particularly against malaria, which causes more than 500 million cases annually and 2–3 million deaths, mainly in children aged younger than 5 years in sub-Saharan Africa (Snow et al. 2005).

References

Baquero J, Miranda S, Murillo O, Mateus H, Trujillo E, Suárez C, Patarroyo ME, Parra C (2006) Reference-strand-conformational-analysis (RSCA) is a valuable tool in identifying MHC-DRB sequences in three species of *Aotus* monkeys. Immunogenetics (in press)

Cardenas C, Villaveces JL, Suarez C, Obregon M, Ortiz M, Patarroyo ME (2005a) A comparative study of MHC Class-II HLA-DRB1*0401-Col II and HLA-DRB1*0101-HA complexes: a theoretical point of view. J Struct Biol 149:38–52

Cardenas C, Ortiz M, Balbin A, Villaveces JL, Patarroyo ME (2005b) Allele effects in MHC–peptide interactions: a theoretical analysis of HLA-DRB1*0101-HA and HLA-DRB1*0401-HA complexes. Biochem Biophys Res Commun 330:1162–1167

Cardenas PP, Suarez CF, Martinez P, Patarroyo ME, Patarroyo MA (2005c) MHC class I genes in the owl monkey: mosaic organization, convergence and loci diversity. Immunogenetics 56:818–832

Castillo F, Guerrero C, Trujillo E, Delgado G, Martinez P, Salazar LM, Barato P, Patarroyo ME, Parra-Lopez C (2004) Identifying and structurally characterizing CD1b in *Aotusnancymaae* owl monkeys. Immunogenetics 56:480–489

Corper AL, Stratmann T, Apostolopoulos V, Scott CA, Garcia KC, Kang AS, Wilson IA, Teyton L (2000) A structural framework for deciphering the link between I-Ag7 and autoimmune diabetes. Science 288:505–511

Demotz S, Barbey C, Corradin G, Amoroso A, Lanzavecchia A (1993) The set of naturally processed peptides displayed by DR molecules is tuned by polymorphism of residue 86. Eur J Immunol 23:425–432

Dessen A, Lawrence CM, Cupo S, Zaller DM, Wiley DC (1997) X-ray crystal structure of HLA-DR4 (DRA*0101, DRB1*0401) complexed with a peptide from human collagen II. Immunity 7:473–481

Diaz D, Naegeli M, Rodriguez R, Nino-Vasquez JJ, Moreno A, Patarroyo ME, Pluschke G, Daubenberger CA (2000) Sequence and diversity of MHC DQA and DQB genes of the owl monkey *Aotus nancymaae*. Immunogenetics 51:528–537

Diaz D, Daubenberger CA, Zalac T, Rodriguez R, Patarroyo ME (2002) Sequence and expression of MHC-DPB1 molecules of the New World monkey *Aotus nancymaae*, a primate model for *Plasmodium falciparum*. Immunogenetics 54:251–259

Doebele RC, Pashine A, Liu W, Zaller DM, Belmares M, Busch R, Mellins ED (2003) Point mutations in or near the antigen-binding groove of HLA-DR3 implicate class II-associated invariant chain peptide affinity as a constraint on MHC class II polymorphism. J Immunol 170:4683–4692

Favre N, Daubenberger C, Marfurt J, Moreno A, Patarroyo M, Pluschke G (1998) Sequence and diversity of T-cell receptor alpha V, J, and C genes of the owl monkey *Aotus nancymaae*. Immunogenetics 48:253–259

Fremont DH, Hendrickson WA, Marrack P, Kappler J (1996) Structures of an MHC class II molecule with covalently bound single peptides. Science 272:1001–1004

Garcia KC, Degano M, Stanfield RL, Brunmark A, Jackson MR, Peterson PA, Teyton L, Wilson IA (1999) An alphabeta T cell receptor structure at 2.5 A and its orientation in the TCR–MHC complex. Science 274:209–219

Ghosh P, Amaya M, Mellins E, Wiley DC (1995) The structure of an intermediate in class II MHC maturation: CLIP bound to HLA-DR3. Nature 378:457–462

Hennecke J, Carfi A, Wiley DC (2000) Structure of a covalently stabilized complex of a human alphabeta T-cell receptor, influenza HA peptide and MHC class II molecule, HLA-DR1. EMBO J 19:5611–5624

Kersh GJ, Miley MJ, Nelson CA, Grakoui A, Horvath S, Donermeyer DL, Kappler J, Allen PM, Fremont DH (2001) Structural and functional consequences of altering a peptide MHC anchor residue. J Immunol 166:3345–3354

Lee KH, Wucherpfennig KW, Wiley DC (2001) Structure of a human insulin peptide–HLA-DQ8 complex and susceptibility to type 1 diabetes. Nat Immunol 2:501–507

Marsh SG, Parham P, Barber LD (2000) The HLA facts book. Academic, New York

Moncada CA, Guerrero E, Cardenas P, Suarez CF, Patarroyo ME, Patarroyo MA (2005) The T-cell receptor in primates: identifying and sequencing new owl monkey TRBV gene sub-groups. Immunogenetics 57:42–52

Niño-Vásquez J, Vogel D, Rodríguez R, Moreno A, Patarroyo ME, Pluschke G, Daubenberger CA (2000) Sequence and diversity of DRB genes of *Aotus nancymaae*, a primate model for human malaria parasites. Immunogenetics 51:219–230

Patarroyo ME, Cifuentes G, Vargas LE, Rosas J (2004) Structural modifications enable conserved peptides to fit into MHC molecules thus inducing protection against malaria. Chembiochem 5:1588–1593

Patarroyo ME, Cifuentes G, Salazar LM, Espejo F, Alba MP, Bermúdez A (2005) Based on HLA-DRB1* allele binding specificities, striking differences in distance and TCR contacting residue orientation can be observed in modified protection-inducing malarial synthetic peptides. Curr Med Chem 12:2849–2865

Pico de Coaña Y, Rodriguez J, Guerrero E, Barrero C, Rodriguez R, Mendoza M, Patarroyo MA (2003) A highly infective *Plasmodium vivax* strain adapted to *Aotus* monkeys: quantitative haematological and molecular determinations useful for *P. vivax* malaria vaccine development. Vaccine 21:3930–3937

Rammensee HG, Friede T, Stevanoviic S (1995) MHC ligands and peptide motifs: first listing. Immunogenetics 41:178–228

Reinherz EL, Tan K, Tang L, Kern P, Liu J, Xiong Y, Hussey RE, Smolyar A, Hare B, Zhang R, Joachimiak A, Chang HC, Wagner G, Wang J (1999) The crystal structure of a T cell receptor in complex with peptide and MHC class II. Science 286:1913–1921

Rodriguez R, Moreno A, Guzman F, Calvo M, Patarroyo ME (1990) Studies in owl monkeys leading to the development of a synthetic vaccine against the asexual blood stages of *Plasmodium falciparum*. Am J Trop Med Hyg 43:339–354

Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI (2005) The global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature 434:214–217

Stern LJ, Brown JH, Jardetzky TS, Gorga JC, Urban RG, Strominger JL, Wiley DC (1994) Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. Nature 368:215–221

Suarez C, Patarroyo ME, Trujillo E, Estupiñán M, Baquero J, Parra C, Rodríguez R (2006) Owl monkey MHC-DRB exon 2 reveals high similarity with some humans HLA-DRB lineages. Immunogenetics (in press)