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MHC class II DR classification based on antigen-binding groove natural selection

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ABSTRACT

Major histocompatibility complex (MHC) genes are highly polymorphic and play key roles in immune susceptibility and resistance to pathogens. While the immunological and structural functions of several human and murine alleles have been analyzed, little is known about the MHC molecules of other animals. Here, we could classify five mammalian species into three groups (human, cow and dog, and cat and pig) on the basis of *DRB* nucleotide sequences, synonymous and nonsynonymous mutation rates, and natural selection of individual residues. These observations, along with the locations of the positively and negatively selected residues in three-dimensional DR structures, suggest that the antigen-recognition sites of swine and feline DR molecules have been negatively selected while those of bovine and canine DR molecules have been positively selected. Human DR molecules show evidence of high negative and positive selection. Our observations suggest that MHC-DR molecules are under different selective force depending on each species.

Major histocompatibility complex (MHC) proteins are cell-surface glycoproteins that, within the cell, bind small peptide fragments derived from host and pathogen proteins by proteolysis. The MHC molecules then present these epitopes on the cell-surface, where they are recognized by T cells. This helps the immune system to identify and respond to foreign antigens [1,2]. The peptide-binding sites of MHC I and II molecules are composed of one chain (α chain) and two chains (α and β chains), respectively. X-ray crystallographic analysis of several MHC molecules has revealed that all appear to have a large, outwardly facing, peptide-binding cleft that is formed by two α helixes overlying an eight-strand β-pleated sheet structure. The amino acids that generate this cleft and associate directly with the epitope are called antigen-recognition site (ARS) residues. X-ray crystallographic analysis of the human MHC class II molecule HLA-DR1 bearing an influenza virus haemagglutinin peptide has identified the residues that make up the ARS [3], and a recent study has specified the ARS residues of the DRβ chain to be those occupying positions 9, 11, 13, 26, 28, 30, 37, 47, 57, 61, 67, 70, 71, 74, 78, 85, and 86 [4]. Notably, the ARS of human MHC II molecules is known to be a highly polymorphic site whose variability has been suggested to play a significant role in susceptibility to various diseases, including autoimmune hepatitis [5], rheumatoid arthritis [6], sarcoidosis [7], Vogt–Koyanagi–Harada's syndrome [8], insulin-dependent diabetes mellitus [9], tuberculoid leprosy [10], and severe malaria and hepatitis B virus infections [11]. These observations together show that much is known about the structures and functions of HLA molecules, including their peptide-binding activity and recognition by and activation of T cells. However, it should be noted that these studies on human MHC molecules have tended to focus on specific alleles that appear to be associated with human diseases. It is not clear whether the structural and functional principles derived from these studies are also relevant to other human alleles and the MHC molecules of other mammals.

The diversity of MHC molecules is due to natural selection of the regions within these molecules that are involved in the binding of peptides from various parasites [12]. Since each MHC molecule must protect the host from hundreds of parasites, the part of the MHC molecule that associates with various antigens comes under positive selection that is the process by which new advantageous genetic variants sweep a population. Meanwhile, the MHC molecule regions that are needed to retain its structural integrity are under negative selection that is the process by which new genetic variants sweep out from population. Hence, determining which MHC gene and protein domains are under positive or negative evolutionary selection is important for identifying molecular sites that could be targeted by therapy and developing new vaccines and

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Table 1
Polymorphic amino acid substitutions in *SLA-DRB1*, *FLA-DRB1*, *HLA-DRB1*, *BoLA-DRB3*, and *DLA-DRB1* molecules and their natural selection.

Codon position[a]	*SLA-DRB1*		*FLA-DRB1*		*HLA-DRB1*		*BoLA-DRB3*		*DLA-DRB1*	
	Residue[b]	Selection[c, d]	Residue[b]	Selection[c, d]	Residue[b]	Selection[c, d]	Residue[b]	Selection[c, d]	Residue[b]	Selection[c, d]
9*[e]	DFHYL	N	DFIT	N	EKW	P	EQ		EKRY	P
10	LQM	P	LM		ELQY	P	Y		MQV	
11*	EGLRSV	P	GLW		DGLPSV	P	ACHLRSTY	P	AFLVY	
12	KR	N	KR		KMRT		KQT		K	
13*	ADFHRS		AFGST	P	FGHPRSY	N	GKRS		AFPS	P
14	E		E		EK	N	E		E	
15	C		CS	N	C		C		C	
16	HR		DHR		HQY	P	H		HY	
17	FY		FY	N	F		F		F	
18	F		PT		F		F		T	
19	NST	N	N		N	N	DN		N	
20	G		G		G		G		G	
21	T	N	T	N	T		T		T	
22	ED		E		E		E		E	
23	QR		QR	P	R		R	N	R	
24	ALV		V	N	V		LV		V	
25	LR	N	QRW	N	QR		QR		R	
26*	FLY	N	FLY		FLY		FLY	P	FLY	N
27	LM	N	L		LP		L		LV	P
28*	DELQ		ADEHIRV	P	DEH		DEHN		AEMV	N
29	KR	N	GR	N	RS		R		R	
30*	HNQY	N	CFHY		CGHLRY	P	CHSY	P	DHSY	P
31	CFLY	P	CFSVY	N	FIV		FY		I	
32	Y		HY		HY	P	HTY	P	HY	P
33	N	N	N		HN		N		N	
34	G		GRW	P	QR	N	G		R	
35	DE		E		E		E		E	
36	E		E		E		E		E	
37*	FHIY	P	CFHLNY	P	DFLNSY	P	FHLNRTY	P	FHINY	P
38	LQV	P	ALV		ALV	P	AV		ALV	
39	R		R		HR		R		R	
40	F	N	FLS	N	FY		F		F	
41	D	N	D		D		D		D	
42	S		GNS		S		S		S	
43	D		EK		D		D		D	
44	LV		MV		V		W		V	
45	G		G		GR		DG		G	
46	E	N	EK		E		E		E	
47*	FY	P	FSY		FY	P	FY	P	FY	P
48	RW		R	N	LQRW		QRW		R	
49	AEV		AP		AV		AE		A	
50	V		V	N	AV		LV		V	
51	T		AST	N	MRT		T		T	
52	E	N	E		E		E		E	
53	CFL		LPV		L	N	L	N	L	
54	G		G		G		G		G	
55	R		QR		R		QRW		R	
56	LP	N	LPR	N	P		PQR		PR	P
57*	DEFSV		DFIT	N	ADISV	N	ADSV	P	DISV	
58	A		AG		AET	N	A		A	
59	KR		EKR		DEG		EKV		E	
60	DNY	P	HY		HSY	P	HLQY	P	SY	P
61*	RWY		LMW	–	W	–	CLW	–	W	–
62	N	N	DN	N	N		N		N	
63	GS		EG	P	S		GS		GPR	P
64	LQR		LQ		Q		Q		Q	
65	K		K		K		K		K	
66	DE		DE		DN		DE		E	
67*	FILP	P	FHIVY	P	FIL	P	FILT	P	FIL	P
68	LM	P	LM		L		L		L	
69	E		DE		E	N	E		E	
70*	DEQ		EGQR		DQR	N	DEQR	P	GQR	
71*	KMRS		ADEGKRST		AEKR	P	AEKR	P	AEGKR	P
72	R		HR	N	AQR	N	R		R	
73	ATE	N	AT		AG	P	A	N	AP	N
74*	AEKSV	N	AE	P	AELQRV	P	AENSVY		AET	P
75	V		V		V		V		V	
76	D		D		D		D		D	
77	T		RTW		NT		RT	P	T	N
78*	AVY	P	FILVY	P	VY	N	VY	P	VY	
79	–	–	CF		C		C		C	
80	–	–	R		R		R		R	
81	–	–	HNRY		H		HY		H	
82	–	–	NS		N		N		N	

Table 1 (*continued*)

Codon position[a]	*SLA-DRB1*		*FLA-DRB1*		*HLA-DRB1*		*BoLA-DRB3*		*DLA-DRB1*	
	Residue [b]	Selection[c, d]	Residue[b]	Selection[c, d]	Residue[b]	Selection[c, d]	Residue[b]	Selection[c, d]	Residue[b]	Selection[c, d]
83	–	–	HY		Y		Y		Y	
84	–	–	G		GR		G		GR	P
85*	–	–	V		AV	P	GV		V	
86*	–	–	DFGSV	P	DGV	N	CFGMV	N	GI	

[a] The codon position numbers follow those of *HLA*.
[b] The standard one-letter code for amino acids was used.
[c] P, positively selected site; N, negatively selected site.
[d] "–" indicates a site that was not assessed.
[e] "*" indicates the putative antigen-recognition site.

peptide therapies. Suzuki and Gojobori [13] have developed a method for detecting the selective force on single amino acids and have used this to identify the positively and negatively selected ARS residues of human and swine class I genes [13,14]. However, the selective forces that operate on the residues of MHC class II molecules are unknown.

Our aim was to identify the polymorphism of each amino acid in the MHC class II molecules of domestic animals (cats, cattle, dogs, and swine) and humans, and examine whether these polymorphisms were driven by positive or negative selection. To detect the selective forces operating on the MHC class II molecule, we compared the rates of positive and negative selection on the DRβ chains of these five species by using the Nei–Gojobori and Suzuki–Gojobori methods. Furthermore, on the basis of three-dimensional (3D) HLA-DR molecule structures, we determined how the natural selection operated on amino acids residues of the ARS in the β chain of the DR molecule. Thus, we here characterize the structural, functional, and evolutionary diversity of the DR molecule of five mammalian species.

Materials and methods

DRB sequences. The β1 domain coding-region sequences employed included 62 swine *SLA-DRB1* sequences (210 bp, amino acid positions 9–78), 96 bovine *BoLA-DRB3* sequences (234 bp, 9–86), 236 human *HLA-DRB1* sequences (234 bp, 9–86), 67 feline *FLA-DRB1* sequences (237 bp, 9–87), and 50 canine *DLA-DRB1* sequences (234 bp, 9–86). These are compiled in the WEB site of the IMGT/HLA and IPD/MHC sequence database (http://www.ebi.ac.uk/). To calculate synonymous (dS) and nonsynonymous (dN) substitution rates in the ARS and non-ARS regions, all β1 domain coding-region sequences were adjusted to the same length (10–78).

Statistical analysis. Relative frequencies of dS and dN substitutions were estimated by using the method of Nei and Gojobori [15] and applying Jukes and Cantor's [16] correction for multiple hits. The MEGA2 program was used to construct the statistical data [17].

Detection of the natural selection of single residues. To detect positive and negative selection of single residues in the β1 domains of human, bovine, feline, swine, and canine DR molecules, we used the method of Suzuki and Gojobori [13]. First, we aligned the 236, 96, 67, 62, and 50 *HLA-DRB1*, *BoLA-DRB3*, *FLA-DRB1*, *SLA-DRB1*, and *DLA-DRB1* alleles, respectively, by using the ClustalX program [18]. These alignments were processed by the adaptsite-d program from the adaptsite 1.3 program package, and the synonymous and nonsynonymous substitution probabilities (*p*-value) at single codon sites were calculated. To estimate the average synonymous and nonsynonymous site numbers, and the total numbers of synonymous and nonsynonymous sites throughout the phylogenetic tree for each codon, we used both the distance-based Bayesian method and the maximum parsimony method to infer the ancestral nucleotide sequences and the Jukes–Cantor model [16] to estimate the number of nucleotide substitutions. Finally, we computed the *p*-value of obtaining the observed values for average numbers of synonymous sites and total numbers of synonymous sites under the assumption of selective neutrality for each codon site using the output of adaptsite-d program.

Structural MHC modeling. We examined the relationship between the adaptsite results and the MHC class II DR molecule structure by using the human MHC-DR1 crystal structure (PDB code: 1DLH) and locating the residues where adaptsite indicated positive or negative selection. The structural modeling was carried out and all pictures of DR were drawn by the graphics software NOC ver. 3.01, which was developed by Nymeyer's group (http://noch.sourceforge.net/).

Results and discussion

Frequency of polymorphic sites in the deduced amino acid sequences of the DRB genes of five species

To examine mammalian *DRB* gene polymorphism, we identified the positions of polymorphic amino acids in the 236 *HLA-DRB1* alleles and compared them to the polymorphic positions in 62, 67, 96, and 50 *SLA-DRB1*, *FLA-DRB1*, *BoLA-DRB3*, and *DLA-DRB1* alleles, respectively (Table 1). In the *HLA-DRB1* sequences, 43 of the 78 amino acid positions (55%) varied while 41/70 (59%), 55/78 (71%), 36/78 (46%), and 25/78 positions (32%) in the *SLA-DRB1*, *FLA-DRB1*, *BoLA-DRB3*, and *DLA-DRB1* sequences varied,

Table 2
The number of positively and negatively selected sites.

Gene	Positive selection (%)	Negative selection (%)	P/N rate
BoLA-DRB3			
ALL	13(16.7)	4(5.1)	3.25
ARS	8(42.1)	1(5.3)	8
Non-ARS	5(8.6)	3(5.2)	1.67
DLA-DRB			
ALL	14(18.0)	4(5.1)	3.5
ARS	9(47.4)	1(5.3)	9
Non-ARS	5(8.6)	3(5.2)	1.67
HLA-DRB1			
ALL	15(19.2)	12(15.4)	1.25
ARS	11(57.9)	4(21.1)	2.75
Non-ARS	4(6.9)	8(13.8)	0.5
SLA-DRB1			
ALL	9(12.9)	17(24.3)	0.53
ARS	6(33.3)	4(22.2)	1.5
Non-ARS	3(5.8)	13(25.0)	0.23
FLA-DRB			
ALL	10(12.7)	16(20.3)	0.63
ARS	6(31.6)	3(15.8)	2
Non-ARS	4(6.8)	13(22.0)	0.31

respectively. This allowed us to divide the five species into two groups: the human (*HLA*), feline (*FLA*), and swine (*SLA*) group, wherein >50% of the residues were polymorphic, and the bovine (*BoLA*) and canine (*DLA*) group, wherein <50% of the residues were polymorphic.

With regard to the 43 polymorphic residues in the 78-residue *HLA-DRB1* segment, 167 polymorphisms were found (2.14 polymorphisms/residue). Of these, 65 were found at 17 positions in the previously defined ARS [4] (3.82 polymorphisms/residue). For *SLA-DRB1*, *FLA-DRB1*, *BoLA-DRB3*, and *DLA-DRB1*, the average variabilities for the same 78-residue segment (only a 70-residue segment was used for *SLA-DRB1 because of the lack of sequence information*) and the defined ARS were 2.19 and 4.01, 2.41 and 4.24, 2.00 and 4.00, and 1.65 and 3.24, respectively. Thus, the ratio of ARS polymorphism to full sequence polymorphism for *HLA-DRB1* (1.79) is almost the same as the ratios for the swine (1.83) and feline (1.76) *DRB1* alleles, but these three ratios are smaller than the ratios for the bovine (2.00) and canine (1.96) *DRB1* alleles.

Frequency of positive and negative selection of single residues in the DRβ molecules

To detect the positive and negative selection of the individual residues in the DR molecules of the five species, we used the same dataset described above and subjected it to the Adaptsite Package Program [13] (Table 1). The numbers and rates of positively and negatively selected residues for each species are summarized in Table 2. In terms of positive selection, the rate estimates revealed high positive selection of the *HLA*, *DLA*, and *BoLA* DRβ1 domains (19.2%, 18.0%, and 16.7%, respectively) and low positive selection of the *FLA* and *SLA* DRβ1 domains (12.7% and 12.9%, respectively). In terms of negative selection, the rate estimates revealed low negative selection of the *BoLA* and *DLA* DRβ1 domains (5.1%), high negative selection of the *SLA* and *FLA* DRβ1 domains (24.3% and 20.3%, respectively), and intermediate negative selection of *HLA* DRβ1 domains (15.4%).

Frequency of positive and negative selection of ARS and non-ARS DRβ molecule residues

Previous studies suggested that differences in MHC function are the result of different selective forces that have led to the ARS being positively selected, while the non-ARS region has been negatively selected or not subjected to selection. Therefore, we asked whether the positive and negative selection rates of the *DRB1* ARS and non-ARS regions of the five species differed (Table 2). Of all five species, the ARS of *HLA-DRB1* had the highest positive selection rate (57.9%). The bovine and canine *DRB* ARSs also had higher positive selection rates (42.1% and 47.4%, respectively) than the SLA and FLA *DRB* ARSs (33.3% and 31.6%, respectively). The ARS negative selection rates were extremely low in cattle and dogs (both 5.3%) but higher in humans, swine, and cats (21.1%, 22.2%, and 15.8%, respectively).

Interestingly, all species studied had equivalent non-ARS positive selection rates (ranging from 5.8% to 8.6%). In contrast, there were large variations in the rate of non-ARS negative selection, with swine and cats having very high rates (25% and 22%, respectively), humans having an intermediate rate (13.8%), and cattle and dogs having low rates (5.2% and 5.2%, respectively).

For each species, we also calculated the P/N rate, which is the number of positive selection sites per negative selection site (Table 2). For the *BoLA* and *DLA* ARSs, there were 8- and 9-fold more positively selected sites than negatively selected sites, respectively. In contrast, for the *SLA* and *FLA* ARSs, there were only 1.5- and 2-fold more positively selected sites than negatively selected sites, respectively. The *HLA* ARS had a more intermediate P/N rate of 2.75.

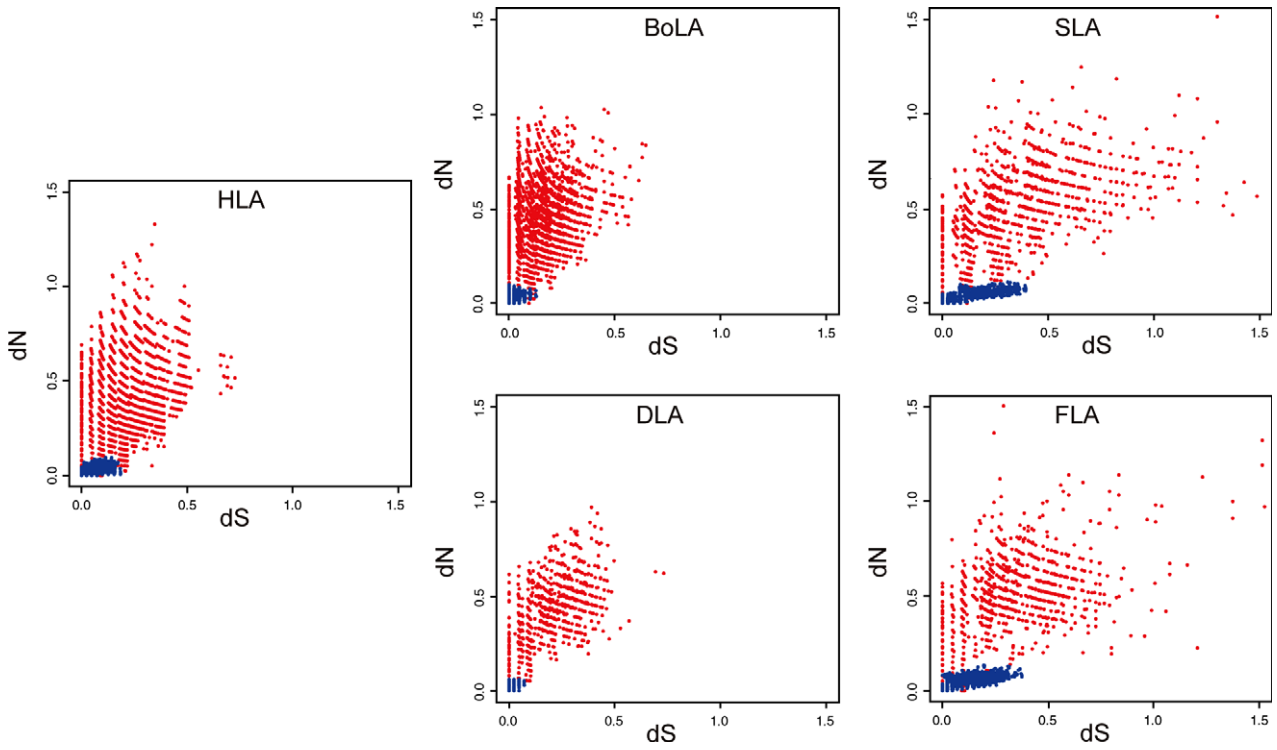

Fig. 1. Comparison of nucleotide substitution patterns of the ARS and non-ARS regions of the β1 domains of swine, feline, human, bovine, and canine DR molecules. To calculate the dS and dN values for the ARSs (red) and non-ARSs (blue), 62 *FLA-DRB1*, 193 *HLA-DRB1*, 49 *DLA-DRB1*, 62 *SLA-DRB1*, and 89 *BoLA-DRB3* alleles were subjected to pair-wise comparisons by using the method of Nei and Goojobori [15,4]. Each symbol is the average value of all pairwise comparisons involving the above-mentioned groups and represents the number of nonsynonymous or synonymous substituted residues relative to the total number of residues in each sequence.

Frequencies of synonymous and nonsynonymous substitutions in the DRB ARSs and non-ARSs

To confirm and visualize the tendency of the ARSs of MHC-DR molecules to be positively selected and their non-ARSs to be negatively selected, we calculated the dS and dN substitution rates for the ARSs and non-ARSs in pair-wise comparisons using 62 *FLA-DRB*, 193 *HLA-DRB1*, 49 *DLA-DRB*, 62 *SLA-DRB1*, and 89 *BoLA-DRB3* alleles. The dS and dN substitution patterns are illustrated in Fig. 1, where dN substitution is plotted against dS substitution for each pair-wise comparison. Because *DRB* alleles were correlated each other, dotted pattern looks like the mackerel sky. For all species, the ARSs had higher dN substitution rates than the non-ARSs. In contrast, the synonymous substitution rates allowed us to divide the five species into three groups. In the first, the ARSs and non-ARSs had moderate synonymous substitution rates (HLA). In the second group, the non-ARSs had small synonymous substitution rates and ARSs had moderate synonymous substitution rates (*DLA* and *BoLA*). In the third group, the ARSs had high synonymous substitution rates and non-ARSs had moderate synonymous substitution rates (*FLA* and *SLA*).

These observations and those shown in Table 2 indicate that ARSs of the bovine and canine DR molecules are more strongly affected by positive selection than the DR molecules of the other animals.

Areas of positive and negative selection in 3D MHC-DR structures

To further compare the DR molecules of the five species, we next examined the positions of the negatively and positively selected single residues in 3D structures. Fig. 2 shows the positively (red) and negatively (green) selected residues in peptide-binding pockets of the MHC-DR molecules of the five species. This suggests that the *BoLA* and *DLA* ARSs were subjected more strongly to positive selection than to negative selection, whereas the *HLA* ARS was subjected to quite high levels of both positive and

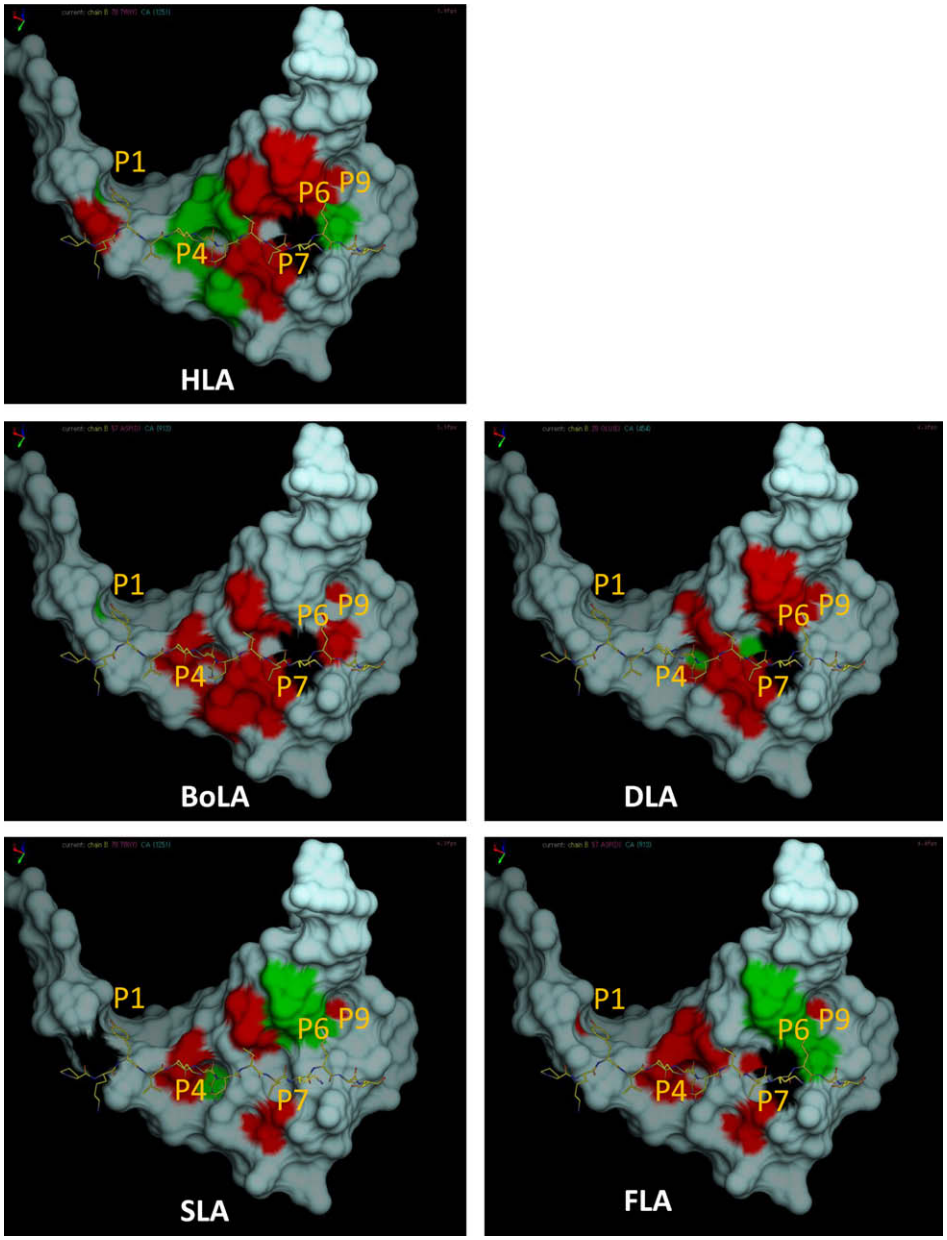

Fig. 2. Comparisons of the position of positively (red) and negatively (green) selected residues in the 3D ARSs of swine, feline, human, bovine and canine DR molecules. Residues that could not be calculated by the adaptsite program are showed as black residues. P1, P4, P6, P7, and P9 indicate the peptide-binding pockets.

negative selection. In contrast, while the *SLA* and *FLA* ARSs were subjected to both positive and negative selection, the selective force was smaller than that on HLA molecules. These results are clearly concordant with the patterns shown in Fig. 1.

MHC-DR structures and classification of the five species according to these structures

Here, we showed for the first time that mammalian *DRB* molecules can be classified into three types on the basis of substitution rates, synonymous and nonsynonymous substitution rates, and type of natural selection. The first group contains the feline and swine DR molecules, which have (1) high and low numbers of polymorphic residues in the entire sequence and ARS, respectively, (2) low and high rates of positive and negative selection, respectively, and (3) small and scattered positively selected areas in the ARS. The second group contains the bovine and canine DR molecules, which have (1) low and high numbers of polymorphic residues in the entire sequence and ARS, respectively, (2) high and low rates of positive and negative selection, respectively, and (3) large positively selected and negligible negatively selected areas in the ARS. The third group contained the human DR molecules only, which had (1) high and low numbers of polymorphic residues in the entire sequence and ARS, respectively, (2) the highest rate of positive selection and a moderate rate of negative selection, and (3) large and concentrated positively and negatively selected areas in the ARS.

We could classify MHC-DR molecules from five mammalian species into three types. It remains to be seen whether MHC-DR molecules from other species are classified into the three types. Also, further study is required to clarify the reason why the selective forces were different among species and to know what are the key factors for determining the selective forces.

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