Immunogenetics (1998) 48:350–353

PEPTIDE MOTIF REGISTER

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HLA-A26 subtype A pockets accommodate acidic N-termini of ligands

Received: 3 February 1998 / Revised: 29 March 1998

Key words HLA · Peptide motif · TFA extraction · Pockets · MHC ligands

The peptide motifs of HLA-A*2601, HLA-A*2602, and HLA-A*2603 molecules were determined by pool sequencing of natural ligand mixtures using established methods (Falk et al. 1991). HLA molecules were immunoprecipitated from C1R cells transfected with the respective genes using W6/32 antibodies (Barnstable et al. 1978), followed by elution of ligands with 0.1% trifluoroacetic acid. Peptide fractionation was performed by reversed phase HPLC on a μRPC C2/C18, 2.1 × 100 mm column (Pharmacia, Uppsala, Sweden) using the SMART System (Pharmacia). Distinct peaks were sequenced directly and the peptide-containing fractions were pooled (dominant peaks were omitted) and sequenced by Edman degradation on a sequencer model 494 A (Applied Biosystems, Weiterstadt, Germany). Evaluation of pool sequencing data was performed as described (Falk et al. 1991; Stevanović 1997).

Pool sequencing of peptide mixtures eluted from each allelic product revealed dominant signals in positions P1, P2, and P9 of ligands. The strongest signals in each peptide pool were caused by valine in P2 and glutamic acid (E) in P1. This E signal (all amino acids are in the one-letter code) in P1 was accompanied solely by D, while no other amino acid was detected above background level. For all alleles investigated here, we found the aliphatic amino acids V, T, I in P2 and, to a lesser extent, L and F. In P9, the aromatic amino acids F and Y were found in ligand mixtures eluted from each allele. Peptide pools from HLA-A*2602 and HLA-A*2603 showed in addition M and L at the C-terminal position. P6, with its preference for hydrophobic amino acids, represents another subtle difference between the three allotypes, since peptide pools from A*2603 had equal amounts of aromatic and aliphatic amino acids, whereas A*2602 preferred aliphatic over aromatic residues, and in A*2601 pools, only aliphatic amino acids were present in significant amounts. While the four positions P1, P2, P6, and P9 were found to be occupied mainly by chemically related amino acids and were therefore classified as anchors or auxiliary anchors, in all other A*26 ligand positions amino acids of various properties were detected. By compiling the data from pool sequence analysis, the peptide motifs shown in Table 1 were defined.

We characterized 11 natural ligands (nine different ones, two occurring twice) bound to the HLA-A*26 subtypes by sequence analysis, and identified their source proteins by data base searching (Table 1). Most of the ligands were nonamers, but peptides of 10 or 11 amino acids were also sequenced. All ligands carry the anchors defined by pool sequence analysis at P2 and the C-terminus, and all ligand sequences but one start with an acidic amino acid in P1. The only exception, a fragment from elongation factor 2, has been found several times among HLA class I-extracted peptides. This peptide therefore represents a promiscuous class I bin-

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Table 1 Natural ligands and peptide motifs of HLA-A*2601, A*2602, and A*2603 molecules. Motif definition was performed as described (Falk et al. 1991; Stevanović 1997). **Bold:** anchors, defined by signal increase in pool sequencing of >100%, and by exclusive occurence in natural ligands. Underlined: auxiliary anchors, defined by signal sequence in pool sequencing of >50% and occurence in a significant number of ligands

HLA-A*2601												
Pos.	1	**2**	3	4	5	6	7	8	**9**			Source
Anchor or auxiliary anchor residues	D E	**V** **T** **I** **F** **L**				I L V M			**Y** **F**			
Preferred residues			I F	P E G	K							
Others			A K D P	N D S	G H K L Q V		A M Q V S	E H K M N Q R				
Ligands	E	**T**	F	N	T	P	A	M	**Y**			β-actin 125–133
	E	**I**	I	G	K	R	G	I	I	G	**Y**	ATP synthase (bovine) 91–101
	Y	**F**	D	P	A	N	G	K	**F**			Elongation factor 2 265–273
	E	**T**	F	G	F	E	I	Q	S	**Y**		zipper containing protein 51–60
HLA-A*2602												
Pos.	1	*2*	3	4	5	6	7	8	**9**			Source
Anchor or auxiliary anchor residues	D E	**V** **T** **I** **L** **F**				I L V F Y M			**Y** **F** **M** **L**			
Preferred residues		Q	I F	P E G								
Others			A K D P Y	Q D S K	G H K L Q R		A M P V S	S H K R N Q				
Ligands	E	**I**	I	G	K	R	G	I	I	G	**Y**	ATP synthase (bovine) 91–101
	D	**I**	P	E	N	V	D	I	T	**L**		60S ribosomal protein L9 11–20
	D	**V**	I	S	S	I	R	N	**F**			(cDNA match)
	E	**I**	K	D	I	L	I	Q	**Y**			40 S ribosomal protein S16 106–114
HLA-A*2603												
Pos.	1	**2**	3	4	5	6	7	8	**9**			Source
Anchor or auxiliary anchor residues	E	**V** **F** **I** **L** **T**				F I L V Y			**Y** **F** **M** **L**			
Preferred residues		Q	I F	E P G	K		I L M	N				
Others	D		M K P D	K Q S D	L Q S		R	A E G				
Ligands	E	**T**	F	G	F	E	I	Q	S	**Y**		Zipper containing protein 51–60
	E	**V**	I	P	Y	T	P	A	**M**			Heme oxygenase 1 103–111
	E	**L**	P	I	V	T	P	A	**L**			Importin α-1 subunit 306–314

der presented by HLA-A*2601, A*3003, B*1516, B*3801, and B*5201 (Falk et al. 1995a, b; Seeger et al. 1998).

In a comparison of the peptide motifs of HLA-A*26 subtypes with other motifs, similarities to several MHC class I alleles become visible. Hydrophobic anchor residues in P2 are demanded by most *HLA-A* alleles, hydrophobic P9 anchors are commonly used among MHC class I ligands, and hydrophobic auxiliary anchors in P6 can often be found. The A*26 motifs are exceptional only in the exclusive usage of acidic amino acids in P1. This phenomenon and the differences between the three subtype motifs can possibly be explained by the pocket structure of these HLA molecules.

In addition, acidic amino acids in P1 are only favored by a few HLA class I peptide motifs such as A*6801, A*6901, A*3303, B*1401, and B*0801 (Barouch et al. 1995; DiBrino et al. 1994; Falk et al. 1994; Guo et al. 1992; Rammensee et al. 1997). (Note that A*3303 replaces the old designation A*3302).

A comparison of the polymorphic residues present in the A pocket of HLA-A26 with other *HLA-A* alleles (Table 2) indicates that α_1 residue 62 is of critical importance for the presence of acidic amino acids in P1. HLA-A molecules harboring such ligands carry R62 and N63, whereas A*0201 for example, which clearly disfavors peptides with acidic P1, has G62 and E63. The positive charge of R62 and the absence of a negative charge in α_1 63 is probably responsible for this selection of ligands: For the 23 HLA-A molecules with R62 and N63, six motifs have been reported and all prefer acidic residues in P1 (Rammensee et al. 1997). This preference might be further enhanced by the presence of another positive charge in the A pocket of A*26 by α_2 residue R163. We thus expect a similar preference of ligands with acidic P1 for A*25 and A*6601, whose motifs have yet to be determined.

Although there is a correlation between P1 residues and pocket A structure in HLA-A molecules, R62 has no impact on restricting peptides with an acidic P1 for binding to HLA-B molecules, since it is present in almost all *HLA-B* alleles with completely different ligand requirements. This observation is another indication that there are considerable differences between HLA-A and B molecules, as previously reported for TAP association (Neisig et al. 1996) and pocket B structure/P2 anchor correlation (Barber et al. 1997; Seeger et al. 1998).

Table 2 Polymorphic residues of pocket A in various *HLA* alleles

Residues Alleles	62	63	66	99	163	167	171	Anchors/preferred residues
*A*2601*	R	N	N	Y	R	W	Y	D, E
*A*6901*	R	N	N	Y	T	W	Y	E
*A*0201*	G	E	K	Y	T	W	Y	No E
*A*3303*	R	N	N	Y	T	W	Y	D, E, M
*A*31012*	Q	E	N	Y	T	W	Y	R, K
*A*0101*	Q	E	N	Y	R	G	Y	No stringency
*B*0801*	R	N	I	Y	T	W	Y	E and others
*B*1401*	R	N	I	Y	T	W	H	D, E, and others
*B*2705*	R	E	I	Y	E	W	Y	K, R, and others
*B*5801*	G	E	N	Y	L	W	Y	K, R, I, and others
*B*4006*	R	E	I	Y	E	W	Y	R, G, and others
*B*0702*	R	N	I	Y	E	W	Y	A, R

The three HLA-A*26 molecules analyzed in this report differ at residues 74, 76, 77, and 116 (Table 3) which may interact with the C-terminal part of ligands. A*2601 and A*2602 are identical in D74, A76, and N77 but differ in position 116, which is D116 in A*2601 and N116 in A*2602. A*2603 also has D116 but differs from both other subtypes by H74, V76, and D77.

The three motifs reported here differ slightly in the P6 auxiliary anchor and in the C-terminal anchor P9. In Table 3, the polymorphic residues of a number of *HLA* class I alleles are listed in order to compare pocket F composition with P9 anchor requirements. There are, however, no visible correlations between C-terminal anchor residues and pocket F structure, neither according to composition analysis (there is no other pocket F composition identical to one of the A*26 subtypes) nor by comparison of distinct pocket amino acids. The same is true for pocket C, which accommodates the P6 auxiliary anchor residues (data not shown).

Table 3 Polymorphic residues of pocket F in various *HLA* alleles

Residues Alleles	70	74	76	77	80	81	95	97	114	116	142	143	147	Anchors/preferred residues
*A*2601*	H	D	A	N	T	L	I	R	Q	D	I	T	W	Y, F
*A*2602*	H	D	A	N	T	L	I	R	Q	N	I	T	W	Y, F, M, L
*A*2603*	H	H	V	D	T	L	I	R	Q	D	I	T	W	Y, F, M, L
*A*31012*	H	D	V	D	T	L	I	M	Q	D	I	T	W	R
*A*3303*	H	D	V	D	T	L	I	M	Q	D	I	T	W	R
*A*0301*	Q	D	V	D	T	L	I	I	R	D	I	T	W	K, Y, F
*A*0201*	H	H	V	D	T	L	V	R	H	Y	T	T	W	V, L
*A*0101*	H	D	A	N	T	L	I	I	R	D	I	T	W	Y
*A*2902*	Q	D	A	N	T	L	I	M	R	D	I	T	W	Y, (L)
*B*27052*	K	D	E	D	T	L	L	N	H	D	I	T	W	L, F, (Y, M, I, R, H, K
*B*1516*	S	Y	E	N	I	A	W	R	D	S	I	T	W	I, V, Y, F, M
*B*5801*	S	Y	E	N	I	A	I	R	D	S	I	T	W	F, W, (Y)

Thus, the three A*26 motifs determined here are rather similar; their common pocket characteristics can be explained reasonably well by their structure, whereas the differences in fine specificity of these three motifs cannot be explained by differences in pocket structure and would not have been predicted.

Acknowledgments This work was supported by grants from the Deutsche Forschungsgemeinschaft (Leibnizprogramm Ra 369/4–1), the Bundesminister für Bildung, Wissenschaft, Forschung und Technologie, and the European Union (Biomed, CT-95–1627), and Grant-in-Aid for Scientific Research on Priority Area from the Ministry of Education, Sport, Culture, and Science of Japan.

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