

Gema Díaz · Benito Cañas · Jesús Vazquez ·
César Nombela · Javier Arroyo

Characterization of natural peptide ligands from HLA-DP2: new insights into HLA-DP peptide-binding motifs

Received: 23 July 2004 / Revised: 14 October 2004 / Published online: 24 November 2004
© Springer-Verlag 2004

Abstract Although natural peptide ligands of HLA-DR and HLA-DQ molecules have been extensively studied, information about peptides naturally bound to HLA-DP is limited. Here we describe HLA-DP2 peptide ligands corresponding to 24 different source proteins that were identified by peptide pool elution and mass spectrometry sequencing from HLA-DP2 molecules expressed on EBV-LCLs. Sequencing analysis led to the identification of both promiscuous and allele-specific peptides. Moreover, the alignment of the natural ligands for HLA-DP2 described here, combined with previous results from our group and others concerning HLA-DP2 antigen presentation and HLA-DP molecular modelling, provide a better understanding of HLA-DP2 peptide-binding motifs.

Keywords HLA-DP antigens · Polymorphism · Peptide-binding · HLA ligand · Peptide motifs

The characterization of HLA peptide ligands and allelic peptide-binding motifs has received considerable attention over the past few years. Autoimmunity, disease resistance, transplantation and vaccine development are some of the areas in which the characterization of natural ligands can help in the design of novel prophylactic vaccines and immunotherapeutic strategies (Hunziker et al. 2001). The sequencing of MHC class I- and class II-bound naturally processed peptides has permitted the characterization of primary anchor residues and the definitions of peptide motifs for many HLA isotypes (Falk et al. 1994; Rammensee et al. 1995). In particular, in the case of class II eluted peptides, pool sequencing and alignment approaches need to analyse large binding-peptide repertoires in order to be effective, or, even better, to combine the alignment of natural ligands with the consideration of predicted pocket structures (Friede et al. 1996; Sanjeevi et al. 2002) and peptide-binding studies (Chicz et al. 1992, 1997; Khalil-Daher et al. 1998).

As reflected in the HLA ligand/motif database that has recently been created (<http://hlaligand.ouhsc.edu>; Sathiamurthy et al. 2003), HLA-DP has been little studied in comparison with other classical HLA molecules. Although 107 HLA-DP alleles have already been characterized (IMGT/HLA database, <http://www.ebi.ac.uk/imgt/hla/index.html>), peptide ligand sequences have been described only for seven of them (<http://hlaligand.ouhsc.edu>). In contrast to the 885 peptide ligands described for HLA-DR, to date only 65 HLA-DP ligands have been identified, probably because of the difficulty involved in collecting sufficient amounts of purified HLA-DP protein (Chicz et al. 1997). The scant information available about HLA-DP contrasts with the association of this molecule with several autoimmune disorders. In particular, HLA-DP2 is one of the HLA-DP molecules that has been related to diseases such as juvenile chronic arthritis (Paul et al. 1993), juvenile rheumatoid arthritis (Begovich et al. 1989), Graves' disease (Dong et al. 1992), berylliosis (Richeldi et al. 1993) and hard metal lung disease (Potolicchio et al. 1999).

G. Díaz · C. Nombela · J. Arroyo (✉)
Departamento de Microbiología II, Facultad de Farmacia,
Universidad Complutense de Madrid,
28040 Madrid, Spain
e-mail: jarroyo@farm.ucm.es
Tel.: +34-91-3941746
Fax: +34-91-3941745

B. Cañas · J. Vazquez
Centro de Biología Molecular Severo Ochoa, Consejo Superior
de Investigaciones Científicas, Universidad Autónoma de
Madrid, Cantoblanco,
28049 Madrid, Spain

G. Díaz
Present address: Facultad de Ciencias de la Salud, Universidad
Rey Juan Carlos,
28922 Madrid, Spain

B. Cañas
Present address: Facultad de Ciencias Químicas, Universidad
Complutense de Madrid,
28040 Madrid, Spain

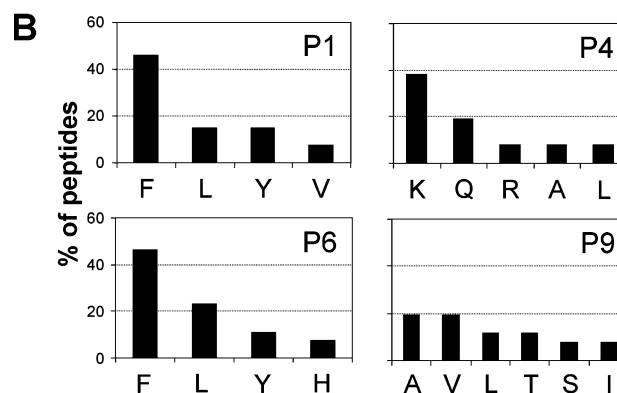
Studies investigating the pool sequences of natural peptides eluted from HLA-DP molecules (Chicz et al. 1997; Falk et al. 1994; Verreck et al. 1994) have afforded the definition of putative anchor residues. Data concerning putative HLA-DP2 peptide-binding motifs (Fig. 1a) were first reported using the alignment of three endogenous peptide sequences (Rammensee et al. 1995; Rotzschke and Falk 1994). Some years later, HLA-DP2 peptide motifs were redefined (Chicz et al. 1997); the anchor residues identified by these authors (Fig. 1a) were more convincing, because they used 12 natural peptide sequences for the alignment and also carried out binding studies with synthetic peptides. Currently, the HLA ligands/motifs database contains the two different HLA-DP2 peptide-binding motifs proposed by these two groups.

To gain further insight into the HLA-DP2 peptide-binding motifs and to define the peptide-binding properties of this molecule more precisely, we were prompted to analyse larger peptide repertoires naturally bound to this molecule. HLA-DP2 molecules were purified from the EBV-transformed human B-cell line 45.1 (Kavathas et al. 1980). In the presence of lysis buffer (Tris ClH 50 mM, ClNa 150 mM, EDTA 5 mM, Nonident-P40 0.5%, pH 8, plus protease inhibitors), 12×10^{10} cells were lysed and then centrifuged at 100,000 g for 90 min. The HLA-DP2-peptide complexes were purified from the detergent-soluble fraction by using tandem affinity chromatography columns in the following order: Sepharose CL-4B, L243-Sepharose CL-4B (anti-DR), B7/21-Sepharose CL-4B (anti-DP). Peptides were then eluted in the presence of trifluoroacetic acid 0.1%. Eluted peptide mixtures were centrifuged through 10,000-Da cut-off ultrafiltration tubes (Centriprep 10, Amicon, Beverly, Mass.) and collected from the flow-through. HLA-DP protein purification was

verified at this step. The fraction retained in the ultrafiltration tube was treated with trypsin and analysed by MALDI-TOF, revealing the presence of tryptic peptides corresponding to α - and β -HLA-DP2 molecules. The peptide pools from the flow-through were vacuum-concentrated and separated by reverse-phase HPLC in a Micro-Reverse Phase column C2/C18, 2.1×100 mm (Amersham Pharmacia Biotech, Piscataway, N.J.). One-minute fractions were collected within the range of 5–45% acetonitrile gradient and analysed by mass spectrometry. Mass assignments for 300 peptides were carried out by MALDI-TOF, revealing a size distribution consistent with previously studied HLA-DP2, DR and DQ repertoires (data not shown). Sequence determinations of peptides were obtained by analysis of peptide fractions by HPLC-ion trap mass spectrometry performed essentially as described previously (Lopez et al. 2002; Marina et al. 1999), with some modifications. A LCQ Deca XP ion trap (Thermo Finnigan, San José, Calif., USA) was coupled to a Surveyor HPLC system, using a RP18 Thermo Hypersil-Keystone ($180 \mu\text{m ID} \times 15 \times \text{cm}$) microcolumn, operating at a flow rate of 1.5 $\mu\text{l}/\text{min}$. The ion-trap mass spectrometer was programmed to work in the “triple play” mode, that is, an MS scan is made to determine whether peaks are present with an intensity above a predefined threshold, in which case a second ZoomScan and a third MS/MS scan are performed on these peaks, using the “dynamic exclusion” method; only two consecutive MS/MS scans are allowed per ion in order to achieve the widest sequence coverage. Alternatively, the ion trap detector was programmed to perform a continuous sequential operation in MS/MS mode on the doubly and triply charged ions corresponding to the masses previously determined by MALDI-TOF analysis (single ion monitoring mode).

Fig. 1 Proposed HLA-DP2 peptide-binding motifs. **a** Comparison of HLA-DP2 anchor features derived from this work and previously described motifs. **b** Amino acid residues detected with greatest frequency in the main HLA-DP2 anchor positions. Calculations were done including only the longer peptide sequences from each source protein

	Relative position									Reference
	1	2	3	4	5	6	7	8	9	
Anchor residue	FLM VWY				FL MY			IA MV		(Rotzschke, 1994)
	FLV YK		DS QT			YF WV			LVI	(Chicz, 1997)
	FLY V		KQ RAL			FLY TH			AVL TSI	This work



Fragmentation spectra were assigned to peptide sequences using as search engines the Sequest program from the Bioworks package (Thermo Finnigan) or the MS/MS ion search page from MASCOT freely available on the Web (<http://www.matrixscience.co.uk>). SWISS-PROT, NR, FASTA or EST protein databases were consulted with

these programs. To corroborate the results provided by the search programs, MS/MS spectra containing interpretable sequence information were also subjected to either a large-scale, automated, "de novo" sequence interpretation, using the program DeNovoX (Thermo Finnigan), or to a manual interpretation. The following criteria were used for manual

Table 1 Natural HLA-DP2 ligands. Amino acid sequences of individual peptides analysed from the HLA-DP2-bound peptide pool with their respective masses are indicated. Putative anchor residues, based on peptide alignment using Clustal W from EMBL (<http://www.ebi.ac.uk/clustalw/>) are in *boldface*. Sequences identi-

fied by the first time as MHC ligands are in *italics*. The known peptide sequences are classified according to the localization of the source protein within the cell: exogenous, surface membrane or cytoplasmic. *Id no.* corresponds to database sequence identification number

Protein source	Sequence	Residue	Mass	Id N°
Exogenous				
Bovine serumalbumine	DEKK F W G K Y L Y E I AR	153-167	1946,019	P02769
	LGE Y G F Q N A L I V R	421-433	1479,796	P02769
Trypsin (chain A) bovine	SIVHPS Y N S N T L N N D IMLIK	70-89	2273,160	P00760
Surface membrane				
Transferrin receptor	GGEP L S Y T R F S L A RQVDG	4-22	1952,984	P02786
	GGEP L S Y T R F S L A RQVD	4-21	1895,963	
	GEP L S Y T R F S L A RQVD	5-21	1838,942	
	EP L S Y T R F S L A RQVD	6-21	1781,921	
	GGEP L S Y T R F S L A RQ	4-19	1681,868	
	GEP L S Y T R F S L A RQ	5-19	1624,847	
	EP L S Y T R F S L A RQ	6-19	1567,826	
	GEP L S Y T R F S L	5-13	1269,651	
	HLA class I, AW-69(A-28) alpha chain	HQYA Y D G K D Y I A L K	113-126	1684,836
YA Y D G K D Y I A L K		115-126	1419,719	
QYA Y D G K D Y I A L K		114-126	1531,782	
QYA Y D G K D Y I A L KED		114-128	1775,851	
HLA-DR alpha chain	LFRK F H Y L P F L P S TED	169-184	2010,052	Q7YPT8
	FRK F H Y L P F L P S TE	170-183	1781,941	
	FRK F H Y L P F L P S T	170-182	1652,899	
	FRK F H Y L P F L P S TED	170-184	1896,968	
Chemokine receptor 4	YA F L G A K F K T S AQ	302-314	1431,766	P51679
	YA F L G A K F K T S AQH	301-314	1568,825	
Vascular endothelial cell growth factor receptor	SVH I Y D K A F I T V KHRK	325-340	1942,106	P17948
Endothelin-converting enzyme 1	TSS F L D Q R F Q D A DEK	386-400	1786,827	P42892
Macrophage scavenger	ID E I S K S L I S L	173-189	1875,036	O60505
Oxytocinase splice variant 2	ED F L D A R F K T M KKDS	495-508	1830,909	Q9UIQ6
Agrin (hypotetic surface membrane)	APVPA F E G R S F L A F PT	1353-1368	1706,993	O00468
NrCAM protein	LPG L E P F S H Y T L NVR	910-924	1742,925	Q92823
c-Maf-inducing protein	KKI Y K Y K K V L S N PSR	7-21	1852,12	Q8IY22
Cytoplasm				
Alpha-enolase	IAPA L V S K K L N V T EQEK	73-89	1868,087	P06733
	IAPA L V S K K L N V T EQE	73-88	1739,992	
	IAPA L V S K K L N V T EQ	73-87	1610,950	
	APA L V S K K L N V T EQE	74-88	1626,908	
	APA L V S K K L N V T EQ	74-87	1497,866	
Cathepsin L2	GG F M A R A F Q Y V KEN	180-193	1617,784	O60911
ATP synthase beta chain	IQR F L S Q P F Q V A EVF	460-474	1808,97	P06576
Ubiquitin	RLI F A G K Q L E D G RT	42-55	1603,894	P02248
Actin beta	RVPPEEH P V L L T E A P I NPK	95-113	2136,184	Q96B34
RAB9A, RAS oncogen	VTNK F D T Q L F H T I GVE	34-42	1848,951	P24408
	NK F D T Q L F H T I GVE	36-42	1648,835	
Tryptophanyl-tRNA synthetase; interferon-induced protein 53	KNV V K I Q K H V T F NQVKG	249-265	1967,155	P23381
Disulfide isomerase A3 precursor	RVM M V A K K F L D A GHK	282-296	1730,958	P30101
Lactate dehydrogenase A	TPKI V S G K D Y N V T ANSK	74-90	1821,973	P00338
Endoplasmin precursor	RET L Q Q H K L L K V IRKK	448-463	2018,274	P14625
Eukaryotic translation elongation factor 1	MAQ F D A K K F A E T QPK	93-107	1739,881	AAP36704

confirmation of sequences: (1) each of the most intense peaks must be assigned to either a *b*- or a *y*-ion that could be double charged (y^{++} , b^{++}) when produced by a triple-charged parent ion fragmentation; (2) long, consecutive tags of *y*- and *b*-ions must be present, allowing the reading of a partial peptide sequence in both directions; and (3) it should be possible to assign most of the minor peaks to neutral losses from *b*- or *y*-ions as H_2O (-18), NH_3 (-17) or CO (-28).

Here, mass and sequence analyses of 44 HLA-DP2 self peptides derived from 24 different source proteins are reported (Table 1). In six of these source proteins (transferrin receptor, HLA-class I α chain, HLA-DR chain, chemokine receptor, α -enolase and RAB9A) we identified predominant epitopes represented by several length variants of the same protein stretch forming sets of nested peptides varying in 1–6 aa at the C terminal or 1–2 aa at the N-terminal ends (Table 1). Of the 44 peptides sequenced, only three (7%) were derived from exogenous proteins; 36% belonged to cytoplasmic proteins and 56% corresponded to surface membrane proteins (Rammensee et al. 1995). Cytoplasmic proteins included cytosolic proteins (such as α -enolase, tryptophanyl-tRNA synthetase, lactate dehydrogenase A, ubiquitin and actin β), lysosomal proteins (cathepsin 12), ER-Golgi proteins (disulfide isomerase A3 precursor, RAB9A, endoplasmic precursor), mitochondrial proteins (ATP synthase) and ribosomal proteins (eukaryotic translation elongation factor 1 γ). In agreement with the foregoing, previous studies have disclosed that peptides derived from plasma membrane proteins are also predominant in other HLA class II molecules, although peptides derived from autologous proteins located in the ER, the Golgi apparatus, secretory vesicles and the cytosol have also been identified (Muntasell et al. 2002).

A striking feature of the HLA-DP2 peptide ligands sequenced was that many of them were enriched in lysine residues. Charged residues were found at the C terminus in 27 of the 44 HLA-DP2 natural ligands analysed, suggesting a dominant amino acid pattern on the flanking regions of the peptides isolated. The presence of positively (34%) or negatively (27%) charged amino acids at C termini might be due to proteolytic degradation by highly specific charged residue proteases involved in antigen processing. Proteases such as trypsin or endopeptidase Lys-C cleave the C terminus of Lys or Arg, corresponding to the pattern found most frequently in the HLA-DP2 natural ligands described here. Preferences at the N termini were not so clear, although charged amino acids were found at this position fairly frequently. Moreover, scrutiny of the complete sequence of the source proteins from the peptides identified showed that the amino acid residue contiguous to the C termini of the sequenced peptide usually included a charged residue (31%) or Leu (20%).

In this study, both promiscuous and putative allotype-specific self peptides were identified. The set of peptides derived from bovine serum albumin, ubiquitin, transferrin receptor, actin, ATP synthase, class I and class II molecules are examples of promiscuous peptides that

have been found to be associated with almost all MHC molecules (Rammensee et al. 1995). Specifically, transferrin receptor 5–21 and bovine serum albumin 152–170 have been identified previously as HLA-DP2 ligands (Rotzschke and Falk 1994). The fact that these peptides were also identified by us, using different elution and sequencing methods, suggests that these ligands must be present in relatively large amounts. Ligands derived from ubiquitin, HLA class I α chain, HLA-DR α chain, actin β and chemokine receptor 4, containing at least part of the peptide sequence identified in this study, have been found to be mainly associated with HLA-DR and HLA-DQ molecules (Futaki et al. 1995; van de Wal et al. 1997). However, the core-binding region of these promiscuous ligands seems to be different for HLA-DP2. Ligands derived from the same proteins, but with a peptide sequence complete different from the stretch identified here, have been reported to be presented by allelic forms of HLA class I and class II and even mouse MHC molecules. The promiscuous peptides described here should help to gain further insight into the functional role of HLA-DP2, since these ligands exhibit mismatches at anchor positions with respect to other MHC alleles.

Thirteen of the 24 source proteins correspond to proteins described for the first time as source proteins for MHC ligands. The 14 peptides derived from these proteins (Table 1) should be considered putative allotype-specific self peptides for HLA-DP2 molecules.

The 44 naturally HLA-DP2 bound peptide sequences were aligned optimally to fit the putative peptide-binding motifs (Table 1). This alignment showed a preference for ligands with hydrophobic and aromatic residues at pocket 1 (Fig. 1). Our data indicate that Phe is the most frequent amino acid for the P1 residue, although this pocket accepts other hydrophobic residues such Tyr, Leu or Val. This is completely consistent with the anchor residues previously proposed (Chicz et al. 1997) at P1 (Fig. 1a). Using molecular modelling, we have previously described a deep and hydrophilic pocket 1 for HLA-DP2, mainly due to the presence of Gly at residue 84, allowing large hydrophobic residues to fit into it (Díaz et al. 2003). The same amino acid preferences have been reported for pocket 1 of HLA-DP4 molecules (Castelli et al. 2002). This is not surprising, since HLA-DP2 differs from HLA-DP4 by only four amino acids at positions 36, 55, 56 and 69, which are predicted to be located at pockets 4, 6 and 9 but not at pocket 1.

Positively charged residues (Lys, Arg) dominate the P4 anchor of HLA-DP2 (Fig. 1). Combining peptide-binding assays and molecular modelling, we and others have previously described the high affinity of this pocket for positive and polar residues (Arg, Thr, Ser) (Berretta et al. 2003; Chicz et al. 1997; Díaz et al. 2003). The preference for these residues is in agreement with the HLA-DP2 molecular modelling predictions (Berretta et al. 2003; Diaz et al. 2003), where HLA-DP2 displays a negatively charged pocket 4 because of the presence of Glu at position 69. It is important to note that Lys, despite being a positively charged amino acid residue, has not been

reported previously as a P4 anchor (Chicz et al. 1997; Rotzschke and Falk 1994). However, this residue was found to be located at P4 in 38% of the HLA-DP2 peptide sequences described here, representing the most frequent residue at this position. In agreement with previous data (Chicz et al. 1997), Gln was found at P4 in many of the peptides sequenced. Residues Arg, Ala, and Leu were also found to be significantly present at P4, although to a lesser extent.

Similar to pocket 1, pocket 6 of HLA-DP2 is a deep and hydrophobic pocket with a preference for aromatic and aliphatic amino acids. Hydrophobic residues such as Phe and Leu were the most frequent amino acids found in P6. In addition to residues Tyr and Phe previously described (Chicz et al. 1997) as P6 anchor sites, our data suggest new P6 anchors such as Leu, Thr or His. Data collected by us previously demonstrated that residue 11 plays a crucial role in peptide binding and T-cell recognition, mainly determining the shape of this pocket (Díaz et al. 2003). Polymorphic position 11 is occupied by Gly in the HLA-DP2 molecule, resulting in a larger pocket 6, with a high affinity for aromatic or branched aliphatic amino acids. HLA-DP2 and HLA-DP402 exhibit similar binding motifs at P6 (Phe, Leu, Tyr) (Castelli et al. 2002; Díaz et al. 2003). The structural characteristics of pocket 6 appear to be similar in HLA-DP2 and HLA-DP402, since polymorphic positions within this pocket (residues 11 and 69) are occupied by the same amino acids in both alleles. Although to a lesser extent than the above-mentioned residues at P6, we detected the presence of His in some of the ligands analysed. The preference of pocket 6 for this aromatic and hydrophobic residue is in agreement with our previous results (Berretta et al. 2003).

The pattern was less restricted in P9 since hydrophobic (Ala, Val) or polar (Thr, Ser) amino acids were found. However, although several chemical features are allowed in this pocket, most peptide residues found at P9 are small. As well as the previously favoured residues proposed for this pocket (Leu, Val, Ile), our data also implicate residues such as Ala, Thr and Ser at P9. The data from HLA-DP2 molecular modelling indicate that the presence of Asp at residue 55 forms a salt bridge hydrogen bond with Arg76 α , reducing the size of this pocket (Díaz et al. 2003), since small residues are the most favoured.

In conclusion, we have extended the number of previously described natural ligand peptide sequences associated with HLA-DP2 from 15 to 62. The alignment of this high number of peptides, together with previous HLA-DP2 peptide-binding studies HLA-DP2 molecular modelling, has allowed us to gain a better definition of the peptide amino acid preferences for this allele. Our data extend the knowledge about HLA-DP2 peptide motifs. We hope this will contribute to a better understanding of the pathogenesis of HLA-DP2 associated diseases.

Acknowledgements This work was supported in part by the Comision Interministerial de Ciencia y Tecnología (Grant SAF97-0134) and by the Comunidad Autónoma de Madrid (CAM)/Universidad Complutense (Proyectos de Grupos Estratégicos de la CAM-UCM). We would like to thank Joaquín Abián for HPLC experiments and Miguel Sánchez, Massimo Amicosante and Dolores Jaraquemada for helpful suggestions and comments.

References

- Begovich AB, Bugawan TL, Nepom BS, Klitz W, Nepom GT, Erlich HA (1989) A specific HLA-DP beta allele is associated with pauciarticular juvenile rheumatoid arthritis but not adult rheumatoid arthritis. *Proc Natl Acad Sci USA* 86:9489–9493
- Berretta F, Butler RH, Diaz G, Sanarico N, Arroyo J, Fraziano M, Aichinger G, Wucherpfennig KW, Colizzi V, Saltini C, Amicosante M (2003) Detailed analysis of the effects of Glu/Lys beta69 human leukocyte antigen-DP polymorphism on peptide-binding specificity. *Tissue Antigens* 62:459–471
- Chicz RM, Urban RG, Lane WS, Gorga JC, Stern LJ, Vignali DA, Strominger JL (1992) Predominant naturally processed peptides bound to HLA-DR1 are derived from MHC-related molecules and are heterogeneous in size. *Nature* 358:764–768
- Chicz RM, Graziano DF, Trucco M, Strominger JL, Gorga JC (1997) HLA-DP2: self peptide sequences and binding properties. *J Immunol* 159:4935–4942
- Castelli FA, Buhot C, Sanson A, Zarour H, Pouvelle-Moratille S, Nonn C, Gahery-Segard H, Guillet JG, Menez A, Georges B, Maillere B (2002) HLA-DP4, the most frequent HLA II molecule, defines a new supertype of peptide-binding specificity. *J Immunol* 169:6928–6934
- Dong RP, Kimura A, Okubo R, Shinagawa H, Tamai H, Nishimura Y, Sasazuki T (1992) HLA-A and DPB1 loci confer susceptibility to Graves' disease. *Hum Immunol* 35:165–172
- Diaz G, Amicosante M, Jaraquemada D, Butler RH, Guillen MV, Sanchez M, Nombela C, Arroyo J (2003) Functional analysis of HLA-DP polymorphism: a crucial role for DPbeta residues 9, 11, 35, 55, 56, 69 and 84–87 in T cell allorecognition and peptide binding. *Int Immunol* 15:565–576
- Futaki G, Kobayashi H, Sato K, Taneichi M, Katagiri M (1995) Naturally processed HLA-DR9/DR53 (DRB1*0901/DRB4*0101)-bound peptides. *Immunogenetics* 42:299–301
- Falk K, Rotzschke O, Stevanovic S, Jung G, Rammensee HG (1994) Pool sequencing of natural HLA-DR, DQ, and DP ligands reveals detailed peptide motifs, constraints of processing, and general rules. *Immunogenetics* 39:230–242
- Friede T, Gnau V, Jung G, Keilholz W, Stevanovic S, Rammensee HG (1996) Natural ligand motifs of closely related HLA-DR4 molecules predict features of rheumatoid arthritis associated peptides. *Biochim Biophys Acta* 1316:85–101
- Hunziker IP, Zurbriggen R, Glueck R, Engler OB, Reichen J, Dai WJ, Pichler WJ, Cerny A (2001) Perspectives: towards a peptide-based vaccine against hepatitis C virus. *Mol Immunol* 38:475–484
- Kavathas P, Bach FH, DeMars R (1980) Gamma ray-induced loss of expression of HLA and glyoxalase I alleles in lymphoblastoid cells. *Proc Natl Acad Sci USA* 77:4251–4255
- Khalil-Daher I, Boisgerault F, Feugeas JP, Tieng V, Toubert A, Charron D (1998) Naturally processed peptides from HLA-DQ7 (alpha1*0501-beta1*0301): influence of both alpha and beta chain polymorphism in the HLA-DQ peptide binding specificity. *Eur J Immunol* 28:3840–3849
- Lopez JL, Marina A, Alvarez G, Vazquez J (2002) Application of proteomics for fast identification of species-specific peptides from marine species. *Proteomics* 2:1658–1665
- Marina A, Garcia MA, Albar JP, Yague J, Lopez de Castro JA, Vazquez J (1999) High-sensitivity analysis and sequencing of peptides and proteins by quadrupole ion trap mass spectrometry. *J Mass Spectrom* 34:17–27

- Muntasell A, Carrascal M, Serradell L, Veelen PP, Verreck F, Koning F, Raposo G, Abian J, Jaraquemada D (2002) HLA-DR4 molecules in neuroendocrine epithelial cells associate to a heterogeneous repertoire of cytoplasmic and surface self peptides. *J Immunol* 169:5052–5060
- Paul C, Schoenwald U, Truckenbrodt H, Bettinotti MP, Brunnler G, Keller E, Nevinny-Stickel C, Yao Z, Albert ED (1993) HLA-DP/DR interaction in early onset pauciarticular juvenile chronic arthritis. *Immunogenetics* 37:442–448
- Potolicchio I, Festucci A, Hausler P, Sorrentino R (1999) HLA-DP molecules bind cobalt: a possible explanation for the genetic association with hard metal disease. *Eur J Immunol* 29:2140–2147
- Rotzschke O, Falk K (1994) Origin, structure and motifs of naturally processed MHC class II ligands. *Curr Opin Immunol* 6:45–51
- Richeldi L, Sorrentino R, Saltini C (1993) HLA-DPB1 glutamate 69: a genetic marker of beryllium disease. *Science* 262:242–244
- Rammensee HG, Friede T, Stevanović S (1995) MHC ligands and peptide motifs: first listing. *Immunogenetics* 41:178–228
- Sanjeevi CB, Lybrand TP, Stevanović S, Rammensee HG (2002) Molecular modeling of eluted peptides from DQ6 molecules (DQB1*0602 and DQB1*0604) negatively and positively associated with type 1 diabetes. *Ann NY Acad Sci* 958:317–320
- Sathiamurthy M, Hickman HD, Cavett JW, Zahoor A, Prilliman K, Metcalf S, Fernandez VM, Hildebrand WH (2003) Population of the HLA ligand database. *Tissue Antigens* 61:12–19
- Wal Y van de, Kooy YM, Drijfhout JW, Amons R, Papadopoulos GK, Koning F (1997) Unique peptide binding characteristics of the disease-associated DQ(alpha 1*0501, beta 1*0201) vs the non-disease-associated DQ(alpha 1*0201, beta 1*0202) molecule. *Immunogenetics* 46:484–492
- Verreck FA, van de Poel, A, Termijtelen A, Amons R, Drijfhout JW, Koning F (1994) Identification of an HLA-DQ2 peptide binding motif and HLA-DPw3-bound self-peptide by pool sequencing. *Eur J Immunol* 24:375–379