High structural side chain specificity required at the second position of immunogenic peptides to obtain stable MHC/peptide complexes

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Abstract Peptides binding to HLA-A11 contain a hydrophobic or a small polar amino acid at position 2 and a lysine at the carboxy terminus. Synthetic peptides carrying natural and unnatural amino acids in position 2 were used to determine the requirements for formation of stable HLA-A11/peptide complexes. By kinetic analysis we demonstrate that a stereospecific interaction between the side chain residue in position 2 and a subsite of pocket B is required to obtain stable HLA/peptide complexes. This specific interaction is mediated by a methyl group or by an ethyl group bound to the asymmetric C_β atom with the correct configuration. Experiments performed with different peptide sequences suggest that the presence of adequate anchor residues may be sufficient to produce stable HLA/peptide complexes.

Key words: HLA-A11; Immunogenic peptide; HLA/peptide stability; Anchor position

1. Introduction

Cytotoxic T lymphocytes (CTL) play an important role in the control of viral, bacterial and parasitic infections, and can recognize and destroy tumor cells [1]. The molecular targets of their attack are peptide fragments selected for presentation by major histocompatibility complex (MHC) class I molecules [2]. MHC class I molecules are polymorphic glycoproteins able to bind short peptides, 8–10 amino acids long, produced by the intracellular degradation of proteins, and display them on the cell surface for surveillance by CTL. Vaccination with synthetic peptides, corresponding to immunogenic epitopes, or with peptide-pulsed cells was shown to induce specific CTLs against immunogenic viral or tumor-derived epitopes in vivo [3–6].

Molecular details of MHC/peptide association demonstrated that the peptide binding site is localized in a groove formed by the two α-helixes lying across an eight-stranded β-pleated sheet [7,8]. The groove accommodates peptides in an extended conformation, allowing the interaction of the amino acid side chains with six defined pockets, designated A to F, that vary in shape, depth, and chemical composition and are characteristic for each MHC allele. Peptides bound to a given class I molecule show common amino acids at particular positions in the sequence, described as anchor residues, which make specific contacts with the groove of class I molecules [9]. These anchor residues, usually positions 2 and 9 of the peptide sequence, interact with pockets B and F respectively, and are necessary for binding.

We have previously shown that peptides binding to the human allele histocompatibility leukocyte antigen (HLA)-A11 contain a hydrophobic or a small polar amino acid at position 2 (p2) and a lysine at the carboxy terminus [10]. Furthermore, we have characterized the molecular requirements for p2 occupancy of HLA-A11 binding peptides by the use of a new approach based on the evaluation of HLA-peptide stability. This parameter, in contrast with peptide binding experiments performed with isolated HLA molecules, is relevant for determining the functionality of HLA/peptide complexes. By these studies we demonstrated that all peptides capable of forming stable complexes with HLA-A11 molecules carry in position 2 an amino acid with a branched side chain and methyl groups on the C_β atom [11]. This residue may be necessary for binding an hydrophobic site of the B pocket.

In order to evaluate in detail the role of the methyl group in the binding between pocket B of HLA-A11 and peptide anchor position 2 we performed a series of studies using synthetic peptides, carrying natural and unnatural amino acids in position 2.

2. Materials and methods

2.1. Cell lines

The ·174/T2 cell line (T2) was obtained by fusion of the peptide transporter mutant·174 LCL with the T cell line CEM [12]. An HLA-A11 positive subline (T2/A11) was obtained by transfection of a genomic *Hin*dIII fragment containing the HLA-A11 coding sequence [13]. Cell lines were maintained in RPMI 1640 (Gibco) supplemented with 2 mM glutamine, antibiotics, 10% heat inactivated fetal calf serum and 200 μg/ml hygromycin B (Sigma).

2.2. Peptide synthesis

Peptides were synthesized by solid-phase method using a continuous flow instrument with on-line UV monitoring (Milligen, Biosearch 9050). The stepwise syntheses were carried out by Fmoc-chemistry; no special efforts were made to optimize the coupling steps. The Fmoc-MBHA resin was swollen in DMF and packed in the reaction column. Fmoc-amino acids were coupled in a 4-fold excess using diisopropylcarbodiimide in the presence of the HOBt. The Fmoc group was cleaved with 20% piperidine-DMF solution. Protected peptides were cleaved from the resin by treatment with modified reagent B (88% TFA, 5% H_2O, 7% Et_3SiH) and the resulting products were collected by centrifugation and purified by preparative HPLC (purification yield 79–90%).

A different method was developed for the IVT analogue containing a residue of homoserine in position 2. The difficulty in incorporating homoserine into synthetic peptides stems from the well-known ten-

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dency of γ-hydroxyamino acids and their derivatives to form a γ-lactone. The key to this synthesis was the introduction, during the solid-phase procedure, of the dipeptide Fmoc-Hse-Thr-OH prepared by solution method to Boc-Asp-Thr-OtBu, and the subsequent conversion by chemoselective reduction of the side chain carboxylic function [14].

The amino acid sequences of the peptides in single letter code were: IVTDFSVIK (IVT), derived from EBV nuclear antigen-4 aa 416–424 [15]; AIFQSSMTK (AIF), derived from the HIV pol antigen aa 325–333 [10]; YVNVNMGLK (YVN), derived from the HBV nucleocapsid antigen aa 88–96 [16]; and ELNEALELK (ELN), derived from wild-type p53 aa 343–351 [10]. The IVT analogues carrying amino acid substitutions in position 2 are referred to as $[x]^2$IVT.

2.3. Peptide analytical determinations

Homogeneity of the purified products was assessed by analytical HPLC. Structure verification was achieved by elemental and amino acid analyses and MALDI-TOF mass spectrometry. The physicochemical data of synthetic peptides are reported in Table 1. Melting points were determined by a Kofler apparatus and are uncorrected. Optical rotations were determined using a Perkin Elmer 141 polarimeter with a 10 cm Water jacketed cell. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) was obtained using the HPG2025A mass spectra. HPLC analysis was performed on a Bruker liquid chromatograph LC21-C instrument, using a Vydac C 18 218 TP 5415 column (175×4.5 mm, 5 mm particle size) equipped with a Bruker LC 313 UV variable wavelength detector; recording and quantification were accomplished with a chromatographic data processor (Epson computer FX80X7). Analytical determinations were carried out by a gradient made up of two solvents: A, 10% (v/v) acetonitrile in water and B, 60% (v:v) acetonitrile in water, both containing 0.1% TFA. The gradient program used was linear gradient from 0 to 100% B in 25 min at a flow of 1 ml/min. All analogues showed purity greater than 99% following analytical HPLC monitored at 220 nm. Preparative reversed-phase HPLC was carried out with a Water Delta Prep 3000 using a Delta Pack C 18-300 A (30 mm×30 cm, 15 mm, spherical). The gradient used was identical to that of analytical determinations. Chromatography was performed at a flow rate of 30 ml/min. Amino acid analyses were carried out using PITC methodology as the amino acid derivatization reagent (Pico-Tag, Waters-Millipore, Waltham, MA, USA). Lyophilized samples of peptides (50–100 pmol) were placed in heat-treated borosilicate tubes (50×4 mm), sealed and hydrolysed using 200 ml 6 N HCl containing 1% phenol in the Pico-Tag work station for 1 h at 150°C. A Hypersil ODS column (250×4.6 mm, 5 mm particle size) was employed to separate the PITC-amino acid derivatives.

2.4. Detection of peptide binding to HLA-A11 class I molecules by immunofluorescence

Aliquots of 1×10^6 T2/A11 cells were cultured overnight in 1 ml serum-free medium containing 10^{-4} M of the indicated peptides. Cells were then extensively washed and resuspended in PBS. Surface expression of HLA class I molecules was detected by indirect immunofluorescence using the mouse mAb, W6-32, which is specific for HLA-A, -B, -C molecules, AUF5.13, which is specific for HLA-A3 and -A11 class I antigens, and BB7.2, which is specific for HLA-A2 molecules. Data are expressed as mean logarithm fluorescence intensity measured with a FACS analyser [10].

2.5. Detection of HLA-A11/peptide complex stability

Aliquots of 5×10^6 T2/A11 cells were cultured overnight in 5 ml serum-free medium containing 10^{-4} M of the indicated peptides. Cells were then extensively washed, treated with mitomycin C (Sigma) to avoid cell proliferation, divided in aliquots in serum-free medium and maintained at 37°C for kinetic experiments. Surface expression of HLA class I molecules was detected by indirect immunofluorescence using the mouse mAb W6-32, which is specific for HLA-A, -B, -C molecules. Data are expressed as mean logarithm fluorescence intensity measured with a FACS analyser [10].

3. Results

3.1. Association of IVT analogues with HLA-A11 molecules

A previously identified HLA-A11 binding peptide was chosen as a model for these studies. This peptide, IVTDFSVIK (IVT), aa 416–424, derived from the Epstein-Barr virus nuclear antigen 4 (EBNA4), was previously shown to efficiently associate with HLA-A11 molecules since it induced high and stable levels of surface HLA-A11 expression in the HLA-A11 transfected T2 mutant cell line (T2/A11) [10,11].

We synthesized the IVT peptide and a series of synthetic analogues (Table 1 and Fig. 1), differing from the original sequence by single amino acid substitution in position 2 (p2). The p2 amino acids were chosen to evaluate the physico-chemical and steric role of the methyl group on the C_β atom of the side chain in interaction with A11 molecules.

Thr and Ile amino acids have previously been shown to represent p2 residues of peptide sequences that stably associate with HLA-A11; these were used as positive controls in all assays. Both residues present the methyl group on C_β [11]. AlloThr and alloIle carry a C_β with an opposite configuration with respect to Thr and Ile and consequently the methyl group on C_β has a different spatial disposition. In allo-amino acids the hydroxyl group of alloThr and the ethyl group of alloIle occupy the position of the methyl group in the correspondent natural residues. Abu and Hse present hydrophobic and hydrophilic side chains with steric hindrance similar to Thr; Leu presents a hydrophobic side chain with steric hindrance similar to Ile. Abu, Hse and Leu do not present the methyl group on C_β nor C asymmetry in the side chain.

The peptide CLGGLLTMV (CLG), derived from the EBV antigen LMP2 [17], associates with HLA-A2 molecules and has been used as control in all assays.

All peptides were tested for their ability to associate with

Table 1
Physicochemical properties of synthetic peptides

Name	Sequence	M.p. (°C)	$[\alpha]_D^{20}$[a]	t_R[b]	MS [M+H$^+$]	
					Calcd.	Found
IVT	H-Ile-Val-Thr-Asp-Phe-Ser-Val-Ile-Lys-OH	178–180	−19.5	13.8	1022.8	1022.8
$[Thr]^2$IVT	H-Ile-**Thr**-Thr-Asp-Phe-Ser-Val-Ile-Lys-OH	194–197	−29.4	12.3	1024.7	1024.7
$[alloThr]^2$IVT	H-Ile-**alloThr**-Thr-Asp-Phe-Ser-Val-Ile-Lys-OH	183–185	−21.4	12.6	1024.7	1024.7
$[Hse]^2$IVT	H-Ile-**Hse**-Thr-Asp-Phe-Ser-Val-Ile-Lys-OH	199–202	−23,8	13.3	1024.7	1024.7
$[Abu]^2$IVT	H-Ile-**Abu**-Thr-Asp-Phe-Ser-Val-Ile-Lys-OH	205–208	−20.5	13.7	1008.8	1008.8
$[Ile]^2$IVT	H-Ile-**Ile**-Thr-Asp-Phe-Ser-Val-Ile-Lys-OH	227–229	−15.2	14.1	1036.8	1036.8
$[alloIle]^2$IVT	H-Ile-**alloIle**-Thr-Asp-Phe-Ser-Val-Ile-Lys-OH	215–219	−25.2	14.5	1036.8	1036.8
$[Leu]^2$IVT	H-Ile-**Leu**-Thr-Asp-Phe-Ser-Val-Ile-Lys-OH	184–186	−17.3	14.8	1036.8	1036.8
ELN	H-Glu-Leu-Asn-Glu-Ala-Leu-Glu-Leu-Lys-OH	211–214	−9.8	8.9	1058.2	1058.2
AIF	H-Ala-Ile-Phe-Gln-Ser-Ser-Met-Thr-Lys-OH	209–211	−40.7	7.3	1012.1	1012.1
YVN	H-Tyr-Val-Asn-Val-Asn-Met-Gly-Leu-Lys-OH	198–201	−22.9	9.4	1037.2	1037.2

[a]c 1.0, methanol; [b]t_R: retention time in HPLC, see Section 2 for conditions.

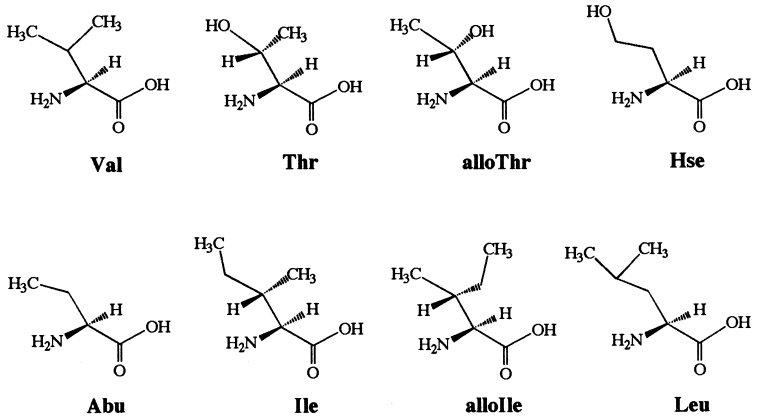

Fig. 1. Schematic representation of stereochemistry of the amino acids at position 2 in IVT peptide analogues.

HLA-A11 molecules expressed at the surface of intact cells. HLA-A11/peptide association was assessed by the induction of surface HLA-A11 expression in the T2/A11 mutant cell line [18]. T2/A11 cells were treated at 26°C for 18 h in serum-free medium to induce high levels of empty molecules at the cell surface [19] and then exposed to 10^{-4} M of peptides at 37° for 1 h. The cells were extensively washed to remove the unbound peptides, treated with brefeldin A to block the egress of new class I molecules, and the surface expression of HLA class I complexes was evaluated by immunofluorescence using the following monoclonal antibodies: W6-32, specific for HLA-A, -B, -C class I molecules, AUF5.13, specific for HLA-A11, and BB7.2 specific for HLA-A2.

Results obtained with the W6-32 and AUF5.13 antibodies showed that the IVT analogues with the amino acids Ile, alloIle, Abu, Hse and Leu in p2 specifically associate with HLA-A11 molecules showing an efficiency comparable to that obtained using the original IVT peptide. An apparent discrepancy between the two detecting antibodies was observed with peptides carrying Thr and alloThr in p2. The HLA-A11/peptide complexes were efficiently detected by W6-32 mAb, but not by AUF5.13 mAb. We have previously shown that this phenomenon is due to the restricted specificity of AUF5.13 mAb for a particular subset of HLA-A11/peptide complexes [11,20]. None of the peptides used was cross-reactive with HLA-A2 molecules, in that they did not affect HLA-A2 expression on T2/A11 (Table 2).

3.2. Stability of HLA/peptide complexes

All the IVT analogues were compared for their capacity to form stable HLA-A11/peptide complexes at the cell surface of T2/A11 cells. After peptide pulsing the cells were extensively washed, mitomycin C treated to avoid proliferation, and tested for HLA-A11 expression in kinetic experiments. HLA-A11 expression was assessed by the use of W6-32 mAb because of its ability to recognize all class I molecules independently of the associated peptide.

Similar levels of HLA-A11 surface expression were ob-

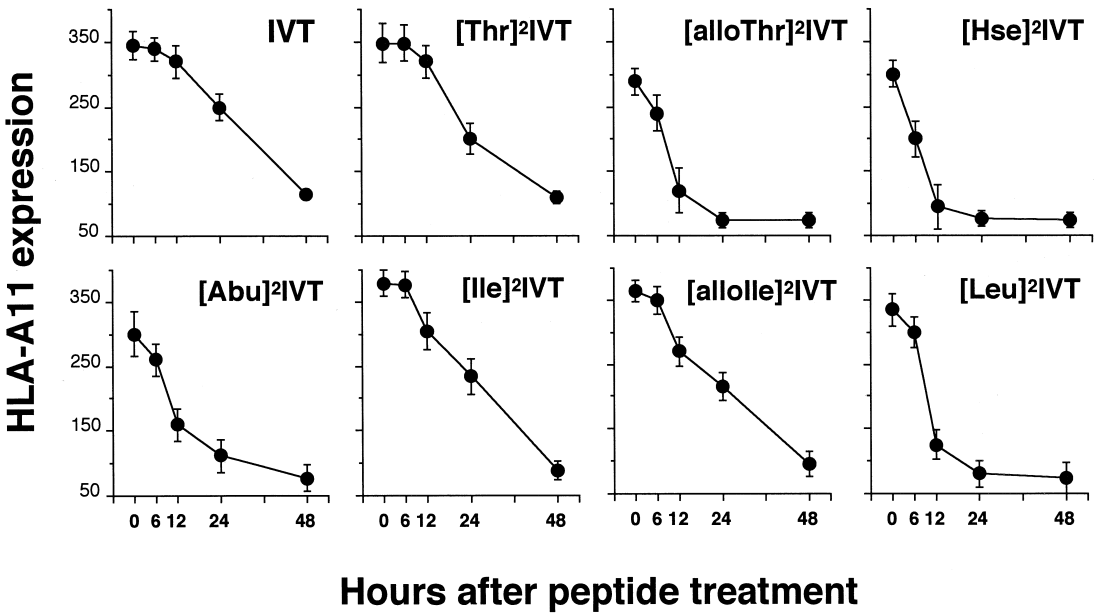

Fig. 2. Kinetic expression of HLA-A11/IVT analogue complexes. T2/A11 cells were preincubated overnight at 37°C with 10^{-4} M of the indicated peptides, washed, divided in 3 aliquots in serum-free medium and maintained at 37°C for kinetic experiments. Surface expression of HLA class I molecules was detected by indirect immunofluorescence using the mouse mAb W6-32. Data are expressed as mean fluorescence intensity measured with a FACS analyser. Mean ± S.D. of 4 different experiments.

Table 2
Induction of surface HLA class I expression by peptides

Peptide	HLA class I expression[a]		
	W6-32	AUF5.13	BB7.2
None	78 ± 12	8 ± 6	18 ± 9
IVT	345 ± 22	65 ± 12	20 ± 4
[Thr]^{2}IVT	348 ± 28	14 ± 4	21 ± 5
[alloThr]^{2}IVT	289 ± 30	14 ± 4	24 ± 6
[Hse]^{2}IVT	300 ± 19	66 ± 10	19 ± 5
[Abu]^{2}IVT	300 ± 24	60 ± 11	19 ± 6
[Ile]^{2}IVT	379 ± 10	64 ± 16	18 ± 6
[alloIle]^{2}IVT	365 ± 18	68 ± 12	18 ± 8
[Leu]^{2}IVT	335 ± 20	63 ± 10	18 ± 5
CLG	280 ± 6	7 ± 8	98 ± 14

[a]Mean fluorescence intensity. T2/A11 cells were incubated overnight in medium alone or medium containing 100 μM of the indicated peptides. HLA class I expression was measured by FACS analysis after indirect immunofluorescence staining with the W6-32 mAb, which is specific for HLA-A, -B, -C molecules, AUF 5.13 mAb, which is specific for HLA-A11 class I antigens, and BB7.2 mAb, which is specific for HLA-A2 molecules. Mean ± S.D. of 4 experiments.

served at time 0 on T2/A11 cells pulsed with the different peptides. T2/A11 cells that were pulsed with the IVT peptide expressed stable levels of HLA-A11/IVT complexes at the cell surface up to 12 h, and the complexes were still detectable over a 48 h period. The [Thr]^{2}IVT, [Ile]^{2}IVT and [alloIle]^{2}IVT analogues showed similar kinetics. In contrast, a net decrease of HLA-A11 expression was observed after just 6 h on T2/A11 cells pulsed with the [alloThr]^{2}IVT, [Hse]^{2}IVT and [Leu]-^{2}IVT analogues, and HLA-A11 expression reached basal levels at 24 h. A similar kinetic behavior was observed with cells treated with the [Abu]^{2}IVT peptide with the exception that detectable HLA-A11 molecules were present at 24 h but completely disappeared at 48 h (Fig. 2).

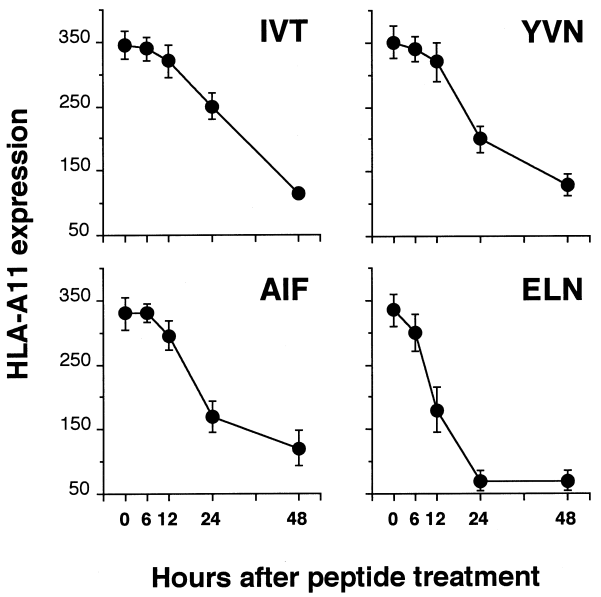

Fig. 3. Kinetic expression of HLA-A11/peptide complexes. T2/A11 cells were preincubated overnight at 37°C with 10^{-4} M of the indicated peptides, washed, divided in 3 aliquots in serum-free medium and maintained at 37°C for kinetic experiments. Surface expression of HLA class I molecules was detected by indirect immunofluorescence using the mouse mAb W6-32. Data are expressed as mean fluorescence intensity measured with a FACS analyser. Mean ± S.D. of 3 different experiments.

3.3. Effect of p2 amino acids in other peptide sequences on HLA-A11 expression

In this set of experiments we evaluated the role of the amino acids in position 2 in unrelated nonamer peptide sequences. HLA-A11 binding peptides of different origin, derived from viral and cellular antigens, were tested for their ability to produce stable complexes with HLA-A11 molecules. T2/A11 cells were treated with 10^{-4} M of YVN, AIF and ELN peptides and the HLA-A11 expression was tested in kinetic experiments.

YVN and AIF peptides, carrying the amino acids Val and Ile at the second position, formed stable complexes with HLA-A11 molecules. In contrast, the ELN peptide, carrying the amino acid Leu, did not stably associate with the A11 antigen (Fig. 3).

4. Discussion

The pool-sequencing analysis of naturally processed peptides presented by a particular HLA class I allele revealed that the majority of these peptides are 9 amino acids long and have specific residues, termed anchors, that allow binding to class I molecules [9]. The molecular interactions between peptides and HLA molecules seem particularly important in determining the type of immunological response; indeed, it has been demonstrated that peptides which stably bind to HLA molecules represent immunodominant targets of CTL activity [21,22].

We have previously shown that the HLA-A11 binding peptides present hydrophobic or small polar residues in position 2 and a lysine in position 9 [10]. In contrast to the strict requirement for lysine in position 9, a large number of amino acids seem to be accepted in the second position. However, only residues with a branched side chain and methyl groups on the C_β atom were able to form stable complexes with HLA-A11 molecules [11]. The p2 amino acid interacts with pocket B of the peptide binding groove, constituted by hydrophobic amino acids at the bottom and polar and charged amino acids at the top [23].

In the present report, a detailed study on the interaction between the methyl group in the anchor position 2 (p2) of the peptide ligand and HLA-A11 class I molecules was performed by analysing peptide binding and the stability of HLA/peptide complexes. We have shown that all IVT p2 analogues are able to associate with HLA-A11, suggesting that pocket B is a complex region that permits multiple interactions with ligands. Indeed, it accommodates both linear and branched residues carrying aliphatic and/or hydroxyl groups.

IVT p2 analogues exhibited a different behavior when their ability to produce stable HLA-A11/peptide complexes was tested. Kinetic analysis, aimed at assessing the stability of complexes for up to 48 h, revealed peptides with the capability to produce stable HLA/peptide complexes.

The [alloThr]^{2}IVT, [Abu]^{2}IVT, [Hse]^{2}IVT and [Leu]^{2}IVT analogues dissociated from HLA-A11 molecules within 24 h of culture at 37°C; on the contrary, the [Thr]^{2}IVT, [Ile]^{2}IVT and [alloIle]^{2}IVT analogues yielded stable complexes, with kinetics comparable to that of IVT.

The different behavior of IVT p2 analogues suggests that at least two interactions between the side chain residue in p2 and pocket B are required to obtain stable HLA/peptide complexes. One of these interactions has to be mediated by a

methyl group (Val, Thr, Ile) or by an ethyl group (alloIle) bound to the asymmetric C_β atom with the correct configuration. The Abu residue presents the methyl group on C_β atom but, independently of the $-CH_3$ spatial disposition, is able to mediate only one interaction with pocket B, whereas two binding sites are required for stability.

From these data it appears that pocket B of HLA-A11 contains a region able to bind polar or non-polar groups, and a specific hydrophobic subsite which mediates interactions exclusively with groups bound to the asymmetric C_β atom of the p2 residue. These interactions may stabilize the HLA/peptide complex either directly or by inducing conformational changes in the bound peptide allowing a more favorable orientation of the N- and C-peptide terminal and/or non-anchor residue groups.

Experiments performed with peptide sequences other than IVT (ELN, AIF, YVN) confirmed the key role of the alkyl group on the C_β atom of the p2 residue, in that AIF and YVN peptides yielded stable complexes while ELN did not. This indicates that the presence of adequate anchor residues is necessary and may be sufficient to produce stable HLA/peptide complexes.

In conclusion, we have shown that the interactions between the anchor positions of peptides and HLA class I molecules are highly specific, and determine the efficiency of presentation of immunogenic peptides.

The precise identification of the role of anchor residues in binding to HLA class I molecules could be particularly useful for the design of highly immunogenic synthetic peptides derived from subdominant epitopes which do not carry proper anchor amino acids. Peptides with increased affinity may be able to enhance stimulation of specific CTL responses directed against natural epitopes. Such high affinity peptides may be useful for CTL-based vaccines and immunotherapy.

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References

[1] Zinkernagel, R.M. and Welsh, R.M. (1976) J. Immunol. 117, 1495–1502.
[2] Townsend, A.R.M., Rothbard, J., Gotch, F.M., Bahadur, G., Wraith, D. and McMichael, A.J. (1986) Cell 44, 959–968.
[3] Schulz, M., Zinkernagel, R.M. and Hengartner, H. (1991) Proc. Natl. Acad. Sci. USA 88, 991–993.
[4] Kast, W.M., Roux, L., Curren, J., Blom, H.J.J., Voordouw, A.C., Meloen, R., Kolakowsky, D. and Melief, C.J.M. (1991) Proc. Natl. Acad. Sci. USA 88, 2283–2287.
[5] Feltkamp, M.C.W., Smits, H.L., Vierboom, M.P.M., Minnaar, R.P., de Jongh, B.M., Drijfhout, J.W., ter Schegget, J., Melief, C.J.M. and Kast, W.M. (1993) Eur. J. Immunol. 23, 2242–2249.
[6] Mukherji, B., Chakraborty, N.G., Yamasaki, S., Okino, T., Yamase, H., Sporn, J.R., Kurtzman, S.K., Ergin, M.T., Ozols, J., Meehan, J. and Mauri, F. (1995) Proc. Natl. Acad. Sci. USA 92, 8078–8082.
[7] Saper, M.A., Bjorkman, P.J. and Wiley, D.C. (1991) J. Mol. Biol. 219, 277–319.
[8] Madden, D.R., Gorga, J.C., Strominger, J.L. and Wiley, D.C. (1992) Cell 70, 1035–1048.
[9] Falk, K., Rötzschke, O., Stevanovic, S., Jung, G. and Rammensee, H.-G. (1991) Nature (Lond.) 351, 290–296.
[10] Zhang, Q.-J., Gavioli, R., Klein, G. and Masucci, M.G. (1993) Proc. Natl. Acad. Sci. USA 90, 2217–2221.
[11] Gavioli, R., Zhang, Q.-J., Marastoni, M., Guerrini, R., Reali, E., Tomatis, R., Masucci, M.G. and Traniello, S. (1995) Biochem. Biophys. Res. Commun. 206, 8–14.
[12] Salter, R. and Cresswell, P. (1986) EMBO J. 5, 943–949.
[13] Masucci, M., Zhang, Q., Gavioli, R., de Campos-Lima, P., Murray, R., Brooks, J., Griffin, H., Ploegh, H. and Rickinson, A. (1992) Int. Immunol. 4, 1282–1293.
[14] Marastoni, M., Salvadori, S., Balboni, G., Scaranari, V., Spisani, S., Reali, E., Traniello, S. and Tomatis, R. (1993) Int. J. Peptide Protein Res. 41, 447–454.
[15] Gavioli, R., Kurilla, M., de Campos-Lima, P.O., Wallace, L., Dolcetti, R., Murray, R.J., Rickinson, A.B. and Masucci, M.G. (1993) J. Virol. 67, 1572–1578.
[16] Tsai, S.-L., Chen, M.-H., Yeh, C.-T., Chu, C.-M., Lin, A.-N., Chiou, F.-H., Chang, T.-H. and Liaw, Y.-F. (1996) J. Clin. Invest. 95, 577–584.
[17] Lee, S.P., Thomas, W.A., Murray, R.J., Khanim, F., Kaur, S., Young, L.S., Rowe, M., Kurilla, M. and Rickinson, A.B. (1993) J. Virol. 67, 7428–7435.
[18] Cerundolo, V., Alexander, J., Anderson, K., Lamb, C., McMichael, A., Goth, F. and Townsend, A. (1990) Nature (Lond.) 345, 449–452.
[19] Ljunggren, H.-G., Stam, N.J., Öhlén, C., Neefjes, J.J., Höglund, P., Heemels, M.-T., Bastin, J., Schumacher, T.N.M., Townsend, A., Kärre, K. and Ploegh, H.L. (1990) Nature (Lond.) 346, 476–480.
[20] Zhang, Q.-J., Lindquist, Y., Levitsky, V. and Masucci, M.G. (1996) Int. Immunol. 8, 927–938.
[21] Sette, A., Vitiello, A., Reherman, B., Fowler, P., Nayersina, R., Kast, W.M., Melief, C.J.M., Oseroff, C., Yuan, L., Ruppert, J., Sidney, J., del Guercio, M.-F., Southwood, S., Kubo, R.T., Chesnut, R.W., Grey, H.M. and Chisari, F.V. (1994) J. Immunol. 153, 5586–5592.
[22] van der Burg, S.H., Visseren, M.J.W., Brandt, R.M.P., Kast, W.M. and Melief, C.J.M. (1996) J. Immunol. 156, 3308–3314.
[23] Guo, H.-C., Madden, D.R., Silver, M.L., Jaerdetzky, T.S., Gorga, J.C., Strominger, J.L. and Wiley, D.C. (1993) Proc. Natl. Acad. Sci. USA 90, 8053–8057.