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MHC class I H-2K^d-restricted antigenic peptides: additional constraints for the binding motif

Gérard Eberl, Alessandra Sabbatini, Catherine Servis, Pedro Romero[1], Janet L. Maryanski[1], and **Giampietro Corradin**

Institute of Biochemistry, University of Lausanne, CH-1066 Epalinges, Switzerland
[1]Ludwig Institute for Cancer Research, Lausanne Branch, CH-1066 Epalinges, Switzerland

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Abstract

The previously defined binding motif of MHC class I H-2K^d-restricted antigenic peptides consists of a Y residue in position P2 and a hydrophobic residue with a large aliphatic side chain (L, I, or V) in position P9/P10 of optimal 9- or 10-mer peptides. We show now that the presence of a charged or a F residue in position P5 reduces the K^d-restricted competitor activity of several cytotoxic T lymphocyte (CTL) epitopes and model peptides, at a degree comparable to A substitutions for the P2 or the P9/P10 anchor residues. Various charged, polar, aromatic, and aliphatic amino acids were substituted for S256 in the CTL epitope *Plasmodium berghei* circumsporozoite (CS) 253–260 8-mer and in its CS 252–260 9-mer form, whereas a more restricted panel of substitutions was tested in the CTL epitopes influenza nucleoprotein 147–155 9-mer and HLA-CW3 170–179 10-mer. Analysis of all the K^d-restricted epitopes so far defined also revealed an uncharged residue at this position. These additional structural constraints present in the K^d binding motif may thus improve the prediction of new epitopes recognized by T cells in the context of this MHC molecule.

The natural class I-restricted antigens were identified as short peptides composed of eight to 12 amino acid residues, and allele-specific peptide motifs were found for several MHC class I molecules (1–8). For example, the H-2K^d-specific motif consists of a Y residue in the second position (P2) relative to the N-terminus, and a hydrophobic residue with a large aliphatic side chain (L, I or V) at the C-terminal end of 9-mer peptides (P9). Studies using peptide analogs of defined K^d-restricted T cell epitopes in functional recognition and competition assays also identified a very similar structural K^d binding motif in optimal 9- or 10-mer peptides (9–11). Since not all of the peptides carrying the specific allele binding motif can interact with the appropriate MHC class I molecule (12–14, unpublished results), additional binding constraints may exist besides the two anchoring residues in terms of amino acid residues allowed to occupy certain positions in the sequence. Here we report that in the case of a number of K^d-restricted epitopes, the presence of a charged or a F residue in position P5 reduced consistently the binding to the K^d molecule, as determined by a functional competition assay. Whether the variations observed are the result of a direct interaction between the class I molecule and the residue in this position or of intrinsic properties of the analog peptides is discussed.

Preliminary competition experiments performed with 8-mer peptide libraries suggested to us that a charged residue in position P5 had a deleterious effect on K^d-restricted competitor activity. This led us to analyze more closely the sequences of the known K^d-restricted antigenic peptides. It is of interest to see that no charged amino acids are present in position P5 in any of the epitopes so far identified, whereas this observation does not hold for the other non-anchor positions (Table 1). We thus performed competition assays with P5 analogs of the 8-mer form of cytotoxic T lymphocyte (CTL) epitope *Plasmodium berghei* (Pb) circumsporozoite (CS) 253–260, shown previously to be only slightly less active than its optimal 9-mer form (10). Various charged, polar, aromatic, and aliphatic amino acid residues were substituted for residue S256 (Table 2 and Fig. 1). The aliphatic- and polar *N*-, Q-substituted analogs were slightly less active (relative competitor activities of 0.04 to 0.8) than the parent peptide. In contrast, the charged E-, R-, K-, and aromatic F-

Correspondence to: G. Corradin

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Table 1. H-2K^d-restricted antigenic peptides

Antigen	Peptide[a]	Sequence[b]	Reference
HLA-CW3	CW3 170–179	R**Y**LKNGKET**L**	10, 17
HLA-A24	A24 170–179	R**Y**LENGKET**L**	10, 17
Influenza NP	NP 147–155	T**Y**QRTRAL**V**	10, 16
P.berghei CS	CS 252–260	S**Y**IPSAEK**I**	10
P.yoelii CS	CS 280–288	S**Y**VPSAEQ**I**	10
P198	P198 14–22	K**Y**QAVTTT**L**	10, 21
Influenza HA	HA 202–212	RTL**Y**QNVGTY**V**	22
(A/NT/60/68)	HA 211–221	**Y**VSVGTST**L**NK	22
Influenza HA (A/JAP/57)	HA 523–545	VYQI LAI **Y**ATVAGS**L**SLI AMMAG	23
MCMV pp89	pp89 167–176	M**Y**PHFMPTN**L**	11
Listeria monocytogenes listeriolysin O	LLO 91–99	G**Y**KDGNEY**I**V	24
P815 P91A/Y13	P91A$^+$ 12–20/Y13	I**Y**TQNRRA**L**	25

[a]All listed antigenic peptides can be recognized by H-2K^d-restricted CTL with the possible exception of the peptide pp89 167–176, which has been originally described as an H-2L^d-restricted epitope and subsequently as an H-2K^d-competitor (11)

[b]The residues corresponding to the H-2K^d-binding motif are in bold characters The amino acid position P5 is underlined.

substituted analogs were poorly active (0.0004–0.002), whereas the charged D-substituted analog showed an intermediate activity (0.02). Since A substitution of the P2 and P9 anchor residues was previously shown to reduce the activity to 0.003 and 0.02 respectively (15), our findings are of quantitative importance. Interestingly, the N and D substitutions gave rise to analogs 15-fold more active than the Q and E substitutions respectively, suggesting a role of the side chain length in the reduction of competitor activity. Moreover, the I and V substitutions, but not the L substitution, also had some deleterious effect (0.04–0.08), suggesting that an even more complex rule accounts for the effect of residue P5 on the CS 8-mer epitope competitor activity.

The R, K, E, and F substitutions showed a less dramatic effect in the context of its 9-mer form PbCS 252–260 (Table 3). The activities were respectively 0.005, 0.03, and 0.1 for the R, K, and E substitutions, whereas the F and D substitutions had only a marginal effect. The comparative observations made for the 8-mer regarding the N and D substitutions and the Q and E substitutions were valid in the case of the 9-mer, whereas no clear effect was observed with the I substitution (the V substitution was not tested).

In order to assess whether our findings were of more general applicability, we examined two additional CTL epitopes: influenza nucleoprotein (NP) 147–155 9-mer (16) and HLA-CW3 170–179 10-mer (17) (Table 4). The latter epitope was of particular interest since seven amino acids separated the two K^d anchor residues. Both peptides showed a reduction in competitor activity similar to that found for the PbCS 252–260 9-mer peptide upon E substitution of residue P5 (0.1). This reduction was more pronounced (0.003–0.01) for the K- and F-substituted analogs (the latter one was tested only in the NP 9-mer), which were thus 10-fold less active than the corresponding PbCS 9-mer analogs. Interestingly, the HLA 10-mer exhibited a behavior similar to the NP 9-mer for the substitutions tested.

Considering all of these results, it appears that the degree of reduction in competitor activity varied according to the CTL epitopes used. For example, the substitution of E, K, and R for S256 in the CTL epitope PbCS 253–260 YIPSAEKI 8-mer resulted in relative competitor activities of 0.0004 to 0.001, whereas activities of 0.1, 0.03, and 0.005 respectively were obtained for its N-terminus extended 9-mer form (SYIPSAEKI). Similarly, in the 8-mer, the substitution of the aromatic F residue for S256 reduced the activity to 0.002, and substitution of the aliphatic V, I, and the polar Q reduced the activity to ~0.06, whereas all of these substitutions had a marginal effect on the

Table 2. Relative competitor activity of PbCS 253–260 peptide analogs

Peptide	Sequence	Experiment A[a]	Experiment B
375	Y I P S A E K I	1	1
G5	– – – N – – – –	0.8	0.8
G7	– – – L – – – –	0.6	0.4
PR3.5	– – – A – – – –	0.6	0.2
G9	– – – V – – – –	0.06	0.08
G4	– – – Q – – – –	0.06	0.06
G8	– – – I – – – –	0 06	0.04
G3	– – – D – – – –	0.015	0.02
G6	– – – F – – – –	0.0015	0.002
G2	– – – E – – – –	0.0008	0.001
G1	– – – R – – – –	0.0006	0 0004
L1	– – – K – – – –	0.0006	0.0004

[a]The competitor activity of the listed peptides was assessed with CTL clone HLA-1-0 4-E9 at an E:T ratio of 5.1 The antigenic peptide HLA-CW3 170–182 was added at a final concentration of 2×10^{-8}M in both experiments The 50% control lysis for the reference peptide 375 (PbCS 253–260) was obtained at 6×10^{-9} M in experiment A and at 4×10^{-9} M in experiment B. The relative competitor activity is calculated as the concentration of the reference peptide 375 required to obtain 50% control lysis divided by that of the corresponding peptides. Peptides were synthetized using the Fmoc, t-Bu strategy as described by Atherton *et al.* (20). All peptides were HPLC purified on a reverse phase column and further subjected to amino acid analysis. All peptides gave the correct theoretical values of amino acid composition.

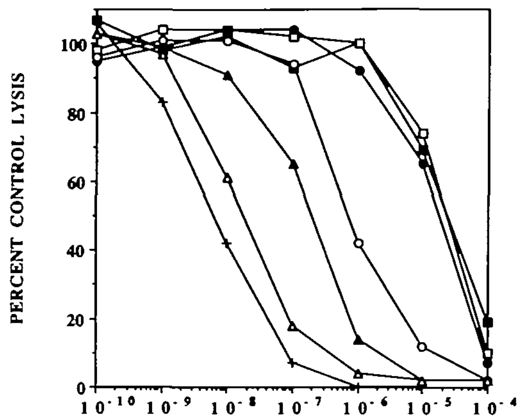

Fig. 1. Titration of the K^d-restricted competitor activity of the PbCS 253 – 260 peptide analogs. ^{51}Cr-labeled P815 target cells were incubated with the indicated final concentrations of competitor peptide. Specified is the position P5 residue in the CS analog. The antigenic peptide HLA-CW3 170 – 182 was added at a final concentration of 2×10^{-8} M and cells from the K^d-restricted CTL clone HLA-1-0.4-E9 were added at an E:T ratio of 5:1. Lysis in the absence of competitor peptides ranged from 60 to 70% and lysis in the absence of antigenic peptide was <5%. The K^d-restricted CTL clone HLA-1-0.4-E9 was derived from BALB/c mice immunized with peptide HLA-CW3 170-182 together with peptide PbCS 57 – 70 (which corresponds to a T helper epitope recognized by CD4$^+$ T cells in H-2^d mice), according to the method described by Widmann *et al* (19). The target cell used in the cytolytic assay is the DBA/2 mastocytoma P815. The competitor activity of the various peptides for the H-2K^d molecule was determined by using the protocol described by Maryanski *et al.* (9) The percent lysis was calculated as: 100 × [(experimental − spontaneous release)/(total − spontaneous release)]. The percent control lysis was calculated as. 100 × (percent lysis with competitor)/(percent lysis without competitor). +, 375 S; ■, L1 K; □, G1 R, ●, G2 E; ○, G3 D; ▲, G4 Q; △, G5 N.

9-mer. The 9-mer form of this epitope was thus more permissive to position P5 substitutions than the 8-mer. The effect of charged residue substitutions in the CTL epitopes influenza NP 147 – 155 9-mer (TYQRTRALV) and HLA-CW3 170 – 179 10-mer (RYLKNGKETL) was either intermediate or similar to those found for the CS 9-mer analogs, depending on the charged residue used.

The side chain length of the particular residues used in the various P5 analogs is a factor in the measured reduction of competitor activity. In all of the peptides tested, the N and D analogs were more active than the Q and E analogs respectively (the latter residues being longer by a methyl group). Moreover, the decrease in activity associated with the basic residue substitutions was, in general, more dramatic than that obtained with the shorter acidic residue substitutions. This difference might be due either to the length of the different side chains or, of course, to the opposite charge of the substituted residues. No such simple correlation can be drawn for the aliphatic residues, since in the CS 8-mer, the A analog was 10-fold more active than the V or I analog, but no difference was observed when the L analog was used. The observation that F substitution reduces the activity of all the peptides tested supports the more general idea that in addition to the charge of the residue P5, the side chain length and size may also have a deleterious effect on the K^d-restricted competitor activity. On the other hand, it should be noted that the A substitution for the F residue occuring in the corresponding position in the epitope MCMV pp89 (11) did not increase its competitor activity. As discussed above, this may be considered an example of sequence dependent variation of peptide analog competitor activity.

At least two simple interpretations could explain the observed data. Amino acid substitution may either change the free energy of the unbound peptides, or that of the peptide – K^d complex,

Table 3. Relative competitor activity of PbCS 252 – 260 peptide analogs

Peptide	Sequence	Experiment A[a]	Experiment B
359	S Y I P S A E K I	1	1
381B	- - - - N - - - -	1	1
381A	- - - - A - - - -	0.9	1
396E	- - - - L - - - -	0.6	0.4
396A	- - - - I - - - -	0.5	0.3
396G	- - - - D - - - -	0.4	0.4
396F	- - - - Q - - - -	0.4	0.2
396D	- - - - F - - - -	0 25	0.3
396B2	- - - - E - - - -	0.1	0.15
381D	- - - - K - - - -	0.04	0.03
396C	- - - - R - - - -	0.006	0.004

[a]The competitor activity of the listed peptides was assessed with CTL clone HLA-1-0 4-E9 at an E:T ratio of 5:1. The antigenic peptide HLA-CW3 170 – 182 was added at a final concentration of 2×10^{-8} M in both experiments. The 50% control lysis for the reference peptide 359 (PbCS 252 – 260) was obtained at 5×10^{-9} M in both experiments. The relative competitor activity was calculated as described in the footnote to Table 2.

Table 4. Relative competitor activity of influenza NP 147 – 155 and HLA CW3 170 – 179 peptide analogs

Peptide	Sequence	Experiment A[a]	Experiment B
W9	T Y Q R T R A L V	1[b]	1
390B	- - - - N - - - -	2.1	2.8
390A	- - - - A - - - -	1.4	1
390C	- - - - E - - - -	0.1	0.09
390E	- - - - F - - - -	0.015	0 01
390D	- - - - K - - - -	0.007	0.009
		Experiment C[a]	Experiment D
382A	R Y L K N G K E T L	1[b]	1
382B	- - - - A - - - - -	2.5	1
382C	- - - - E - - - - -	0 1	0.1
382D	- - - - K - - - - -	0.004	0.003

[a]The competitor activity of the NP 147 – 155 peptide (W9) analogs was assessed with CTL clone HLA-1-0.4-E9 at an E:T ratio of 5:1. The antigenic peptide HLA-CW3 170 – 182 was added at a final concentration of 2×10^{-8} M in both experiments A and B. The competitor activity of the HLA-CW3 170 – 179 peptide (382A) analogs was assessed with CTL clone PbCS QB7.3.2 at an E:T ratio of 5:1. The antigenic peptide PbCS 252 – 260 was added at a final concentration of 1×10^{-9} M in both experiments C and D. The relative competitor activity was calculated as described in the footnote to Table 2. Clone PbCS QB7.3.2 was obtained by immunizing BALB/c mice with peptide PbCS 251 – 260 and a helper epitope as described in the footnote to Fig. 1.

[b]The 50% control lysis for the reference peptide W9 (influenza NP 147 – 155) was obtained at a concentration of 2×10^{-8} M in experiment A and at 2×10^{-7} M in experiment B. For the reference peptide 382A (HLA-CW3 170 – 179), it was obtained at a concentration of 5×10^{-8} M in experiment C and at 2×10^{-7} M in experiment D.

or a combination of the two. In the first case, the difference in activity would be mainly due to a change in the intrinsic properties of the various analogs, i.e. solvation, induced dipolar moment, a particular three-dimensional structure preference. In the second case, no drastic variations of the unbound peptide free energy would occur in the different analogs, but they would interact differently with the class I molecule. For example, the presence of a long charged peptide side chain may clash with some residues present in one of the two class I molecule α helices.

These data may also be relevant to peptides binding to other MHC class I molecules where the position P2 residue has been defined as an anchor residue. To this effect, it is interesting to note that not only none of the K^d-restricted antigenic peptides, but also none of the seven peptides recently eluted from HLA-Aw68 and sequenced contained a charged residue in the position P5 studied here (7). The same observation holds for the 10 self peptides eluted from H-2L^d (18) and the seven described synthetic malaria peptides or self peptides binding to HLA-B53 (14), while only three out of 24 HLA-A2 binding peptides have a charged residue in this position (13).

In conclusion, since the nature of the residue in position P5 affected the K^d-restricted competitor activity of peptide analogs at a degree comparable to A-substitution for the P2 or the P9 anchor residues, these additional binding constraints may improve the degree of confidence in the prediciton of K^d and eventually other MHC class I-restricted epitopes, and the understanding of the molecular basis of the peptide – MHC class I interaction.

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Abbreviations

CS	circumsporozoite
CTL	cytotoxic T lymphocyte
NP	nucleoprotein
Pb	*Plasmodium berghei*

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