Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7871–7875, September 1992
Immunology

Induction of cytotoxic T lymphocytes with peptides *in vitro*: Identification of candidate T-cell epitopes in human papilloma virus

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Communicated by Avrion Mitchison, May 13, 1992

ABSTRACT **A set of overlapping peptides corresponding to the L1, E6, and E7 proteins of human papilloma virus 16 was tested for their ability to bind to major histocompatibility complex class I molecules and to stimulate cytotoxic T-lymphocyte (CTL) responses *in vitro*. A class I binding assay using intact RMA-S cells showed that 20 of the 99 human papilloma virus peptides bound to H-2K^b and/or D^b molecules. Fifteen of the 20 class I-binding peptides stimulated primary CTL responses, whereas peptides that were negative in the binding assay failed to do so. Peptide-induced CTLs recognized the immunizing peptide very efficiently, requiring no more than 1–10 nM peptide for target cell lysis. However, two observations were made that have important implications for the design of peptide-based vaccines for inducing CTLs. (*i*) Not all major histocompatibility complex-binding peptides that contained known motifs characteristic of naturally processed peptides induced CTLs. (*ii*) The efficiency of CTL lysis was strongly decreased when the size of the target peptide differed by only one amino acid residue from that of the immunizing peptide. We conclude that peptides chosen for vaccination must correspond in length to naturally processed peptides.**

Major histocompatibility complex (MHC) class I molecules bind peptide fragments derived from intracellular proteins and present them on the cell surface to cytotoxic T lymphocytes (CTLs). However, the number of epitopes recognized by CTLs in a foreign protein appears to be very limited. For example, the CTL response of H-2^b mice infected with influenza virus was found to be always directed against residues 366–374 of the nuclear protein and no other CTL epitopes were detected (1). Similarly, the CTL response of these mice to vesicular stomatitis virus was directed exclusively against residues 52–59 of the nucleocapsid protein (2). The mechanism of this epitope selection in anti-virus CTL responses is currently not well understood. In particular, it is unknown whether class I molecules can bind only a few virus-derived peptides or whether only a few of many class I-bound peptides stimulate a CTL response.

Human papilloma virus (HPV) 16 is an oncogenic DNA virus associated with a high percentage of neoplastic cervical lesions (3, 4). The aim of this study was to identify MHC class I-binding and CTL-inducing peptide epitopes in the viral E6, E7, and L1 proteins, without using predictive motif sequences. To do this, sets of overlapping synthetic peptides were screened for binding to MHC class I molecules expressed on the surface of mutant RMA-S cells (5) and tested for induction of peptide-specific CTLs *in vitro*. This approach will aid the rational design of peptide-based vaccines for the control of HPV infection.

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MATERIALS AND METHODS

Peptides. Peptides spanning the sequences of HPV16 E6 and E7 proteins (10-mers overlapping by 5 amino acids) and of the L1 protein (15-mers overlapping by 5 amino acids) were synthesized in batches of 25 on a DuPont R^{a}MPS multiple peptide synthesis apparatus using conventional solid-phase fluoren-9-ylmethoxycarbonyl (Fmoc) chemistry. For all peptides the HPLC profile was determined and, where indicated, HPLC purified fractions were used for experiments. Influenza virus peptides NP-(345–360) and NP-(365–380) were gifts of A. Townsend (John Radcliffe Hospital, Oxford) and the Sendai virus peptide SV-(323–331) was provided by H. Ploegh (The Netherlands Cancer Institute, Amsterdam). Peptides were dissolved in phosphate-buffered saline (pH 7.4) to give 2 mM stock solutions and stored at −20°C.

Antibodies. Monoclonal antibodies (mAbs) HB11 and Y3 (6) (both anti-K^b) and mAbs HB27 and B22 (7) (both anti-D^b) were used as undiluted culture supernatants. Second-layer antibody was a goat anti-mouse immunoglobulin-fluorescein isothiocyanate conjugate (Becton Dickinson).

Peptide Binding Assay. RMA-S cells (>98% viability by trypan blue exclusion) were cultured in triplicates with 200 μM peptide in RPMI 1640 medium/10% (vol/vol) fetal calf serum in round-bottomed 96-well plates (nontissue culture treated), at a density of 7×10^4 cells per well for 18 h at 37°C in 5% CO_2/95% air. For indirect immunofluorescence, the cells were stained on ice for surface K^b or D^b or with second-layer antibody only. Eight to 10 triplicates of control cells cultured in the absence of peptide were similarly stained. Flow cytometry was performed using a FACScan (Becton Dickinson). FACScan research software was used to determine the mean fluorescence channel (FL1) for each stained sample on a 4-log scale containing a total of 1023 fluorescence channels. To obtain a linear measure for the FL1 intensity, we used the following formula: mean FL1 = $10^{(\text{mean FL1 channel}/1023)} \times 4$.

***In Vitro* Induction of CTLs.** RMA-S cells were cultured at 25°C overnight to induce high levels of MHC class I expression (8). Induced cells were cultured for 3 h at 31°C in the presence of 100 μM peptide and subsequently treated with mitomycin C (50 μg/ml) for 1 h in the presence of 50 μM peptide. After extensive washing, 10^6 of these RMA-S cells were used to stimulate 2×10^7 naive C57BL/10 spleen cells in T25 Falcon tissue culture flasks containing 10 ml of Iscove's modified Dulbecco's culture medium supplemented with 10% fetal calf serum and 10 μM 2-mercaptoethanol. Peptides were added to a concentration of 6 μM. After 5 days, responder lymphocytes were harvested and cultured in 24-well plates at a density of 8×10^5 cells per well. The culture

Abbreviations: CTL, cytotoxic T lymphocyte; HPV, human papilloma virus; MHC, major histocompatibility complex; mAb, monoclonal antibody; FL1, mean fluorescence channel.

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medium was the same as above except that 10% Con A supernatant was added. Each well received 2×10^6 irradiated C57BL/10 spleen cells as feeder cells and peptides were added to a concentration of 6 μM. After 1 week, responder T cells were restimulated for 1 additional week by using the same conditions as described, and then their CTL activity was determined in a 4-h ^{51}Cr release assay using as targets temperature-induced RMA-S cells that were pulse-labeled with either the immunizing peptides or with control HPV peptides that were known to bind H-2^b class I molecules.

RESULTS

Peptide Binding to H-2K^b and D^b Class I Molecules. The H-2^b-derived mutant thymoma cell line RMA-S has a defect affecting peptide loading of MHC class I molecules (9). As a consequence, the cell surface expression of these molecules is low but was shown to increase when K^b or D^b binding peptides were added to the culture medium. We have used this observation to test 99 synthetic peptides derived from the HPV16 proteins E6, E7, and L1 for binding to H-2^b class I molecules. RMA-S cells were cultured overnight in the presence of 200 μM peptide, and the following day cells were stained with mAbs specific for H-2K^b and D^b and levels of expression were determined by FACScan analysis. In each experiment, 8–10 control RMA-S samples cultured without peptides were included to measure the baseline levels of class I expression. As positive controls we used known K^b binding peptides from influenza nucleoprotein [NP-(345–360)] and Sendai virus [SV-(323–331)] or D^b binding peptides [NP-(365–380) and SV-(323–331)] (9, 10). Fig. 1 *A* and *B* shows two representative experiments of the analysis of overlapping E6 peptides and E7 peptides, respectively. E6 peptides E6-(41–50) and E6-(106–115) mediated an increase in the level of K^b expression that was comparable to that of the positive control peptides. A less-significant increase was seen with E6 peptides E6-(31–40), E6-(36–45), and E6-(81–90) (Fig. 1*A*). Similar low levels of fluorescence were induced by some of the E7 decamers. Analysis of the set of overlapping L1 15-mer peptides revealed some peptides that bound as strongly as the positive controls, with other peptides showing only small increases (data not shown). To determine whether small increases were consistent, we then performed five experiments with each set of peptides. Table 1 shows the peptides that gave positive results in five of five experiments. Peptides were scored positive for binding if levels of K^b or D^b increased by >3 SDs from the mean of the 8–10 control RMA-S samples that were cultured in the absence of peptides. Twenty out of the 99 peptides consistently bound to K^b or D^b molecules. For some of these peptides [L1-(91–105), L1-(141–155), L1-(231–245), E6-(41–50), E6-(81–90), and E6-(106–115)], we performed experiments with HPLC-purified fractions to confirm that the main product of the peptide synthesis was responsible for class I binding and not shorter peptide by-products (data not shown). Other peptides induced marginal increases in class I expression in only some, but not all, of the five experiments [for example, K^b binding by L1-(201–215) in Table 1]. Such peptides were considered negative for class I binding in this study.

Primary Stimulation of Peptide-Specific CTLs *in vitro*. To establish conditions for efficient *in vitro* induction of anti-peptide CTLs, we investigated the use of peptide-loaded RMA-S cells as inducers. Experiments with the known D^b-restricted peptide SV-(323–331) (10) showed that peptide-loaded RMA-S cells induced a weak CTL response after 5 days. Upon restimulation with irradiated splenocytes in the presence of 6 μM peptide (without RMA-S cells) the CTL activity increased dramatically, reaching a plateau after 2 weeks (data not shown). Based on these observations we used a 2-week CTL induction protocol to test HPV peptides.

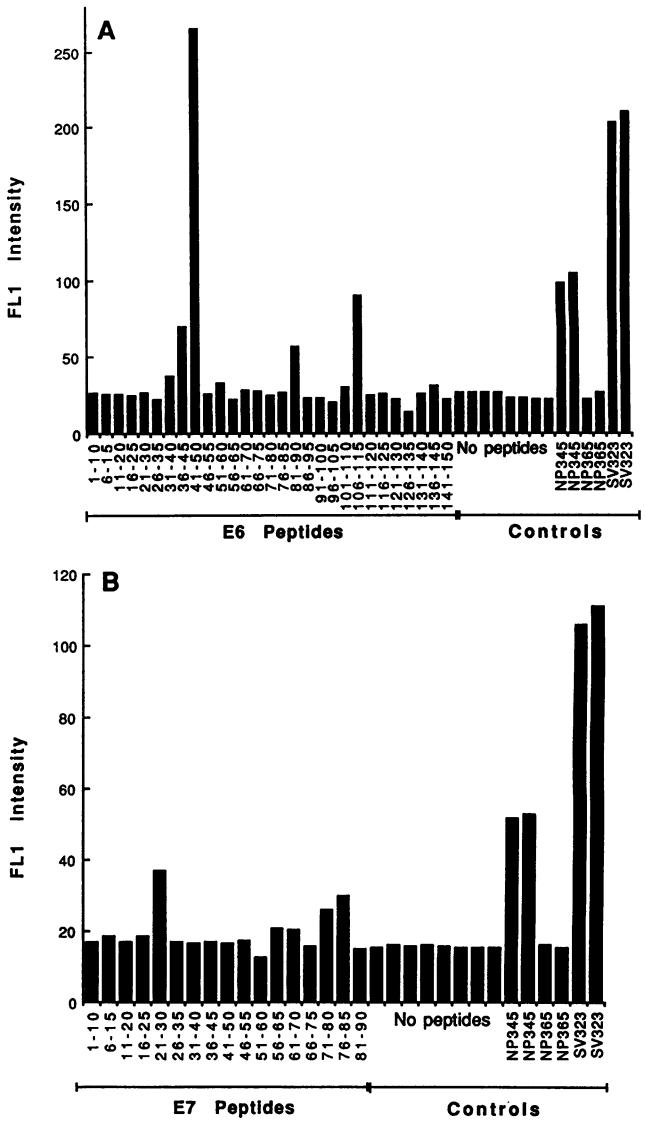

FIG. 1. Peptide-mediated upregulation of K^b expression in RMA-S cells. E6 (*A*) or E7 (*B*) peptides were added to RMA-S cells at 200 μM. After overnight culture, levels of K^b expression were determined by FACScan analysis using K^b-specific mAbs. Sendai and influenza virus peptides were used as positive control peptides [K^b binding, SV-(323–331) and NP-(345–359); D^b binding, SV-(323–331) and NP-(365–379)]. In each experiment, 8–10 RMA-S control samples were cultured in the absence of peptides to determine baseline levels of K^b and D^b expression. HPV peptides that increased levels of K^b or D^b expression >3 SDs of the controls were classed as positive for class I binding and are listed in Table 1.

The results of a typical experiment using four peptides from the E6 protein are shown in Fig. 2. Peptides E6-(41–50) and E6-(81–90) induced good CTL responses, whereas peptide E6-(71–80) failed to do so. Failure of E6-(71–80) to induce CTLs correlated with its lack of binding to K^b (Fig. 1*A*) or D^b (data not shown). Surprisingly, peptide E6-(106–115) only induced a weak CTL response, although it consistently bound strongly to H-2K^b (Fig. 1*A*). To test whether the CTL activity would improve upon further passage, a long-term T-cell line was established. Gradually, this anti-E6-(106–115) line lost all CTL activity and staining after 2 months of culture revealed that 100% of the cells were CD4-positive (data not shown).

Fifteen of the 20 MHC class I binding peptides were found to induce CTL responses (Table 2). Among the noninducing peptides were two that bound strongly [E6-(106–115) and

Table 1. HPV16-derived peptides that increase the levels of K^b and/or D^b expression in RMA-S cells

Type	Peptide	Level of H-2 class I expression	
		K^b	D^b
L1 (15-mer)	L1-(61–75)	38	13
	L1-(71–85)	44	11
	L1-(91–105)	71	11
	L1-(141–155)	177	25
	L1-(161–175)	37	20
	L1-(201–215)	36	26
	L1-(231–245)	117	38
	L1-(251–265)	48	15
	L1-(381–395)	43	42
	L1-(471–485)	38	10
	L1-(481–495)	35	19
	L1-(501–515)	32	10
Control	SV-(323–331)	190	75
	NP-(345–360)	105	14
	NP-(365–380)	26	24
	No peptide	26[a]	10[b]
E6 (10-mer)	E6-(36–45)	69	11
	E6-(41–50)	266	13
	E6-(81–90)	56	15
	E6-(106–115)	89	10
Control	SV-(323–331)	206	80
	NP-(345–360)	101	17
	NP-(365–380)	24	33
	No peptide	24[c]	10[d]
E7 (10-mer)	E7-(21–30)	40	7
	E7-(61–70)	25	6
	E7-(71–80)	25	7
	E7-(76–85)	32	7
Control	SV-(323–331)	133	35
	NP-(345–360)	66	8
	NP-(365–380)	18	13
	No peptide	17[e]	6.3[d]

Approximately 7×10^4 cells were cultured with 200 μM peptide in growth medium for 18 h and stained with mAbs to K^b and D^b molecules. Sendai and influenza virus peptides were used as positive control peptides (see Fig. 1). Peptides listed are those that scored positive for K^b or D^b binding in five of five experiments. Peptides were classed as positive if the increase in K^b or D^b expression was >3 SDs of control RMA-S samples cultured without peptide. Values are the FL1 for K^b and D^b staining of one representative experiment. Underlined values indicate that positive results for K^b or D^b binding were obtained in five experiments. Values for 3 SDs are as follows: a, 4.4; b, 1.8; c, 6.8; d, 1.2; e, 3.6; f, 2.2.

L1-(231–245)] and three that bound weakly [E7-(61–70), E7-(76–85), and L1-(481–495)] to class I molecules (Table 1). None of a panel of five peptides that were negative in the class I binding assay induced specific CTL responses (Table 2), and only a low level of nonspecific lysis was observed.

Specificity and Sensitivity of Peptide-Stimulated CTLs. Several peptide-specific CTLs were established as long-term lines and a line to E6-(41–50) was maintained in culture for nearly 1 year. We first tested whether the sensitivity of anti-HPV peptide CTLs is comparable to that of CTLs generated *in vivo* in other viral systems. It has been shown that half-maximal target cell lysis by anti-influenza virus CTLs required 1–10 nM peptide (11, 12). Analysis of the lysis of a CTL line specific for E6-(41–50) showed that half-maximum lysis required 8–15 nM peptide (Fig. 3 *A* and *B*). This was very similar to the sensitivity of a CTL line specific for the E6-(81–90), which showed half-maximal lysis at 4–8 nM peptide (data not shown).

Since E6-(41–50) was found to bind to K^b but not D^b class I molecules (Table 1), CTL recognition was expected to be

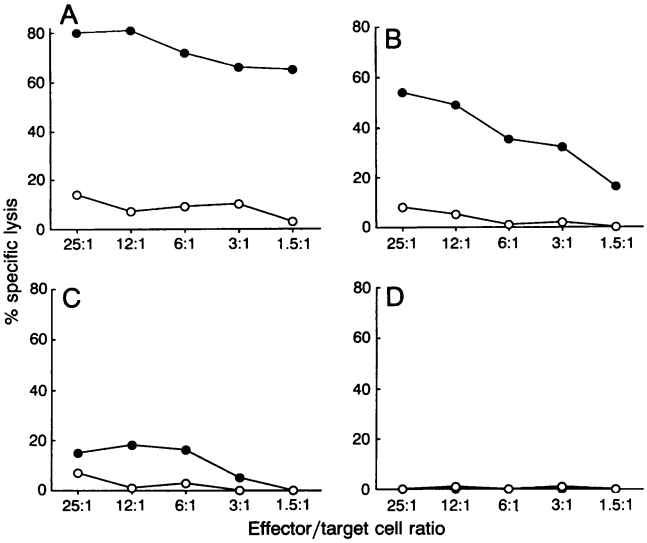

FIG. 2. CTL induction by HPV E6 peptides. Naive C57BL/10 spleen cells were induced *in vitro* with the E6 peptides E6-(41–50) (*A*), E6-(81–90) (*B*), E6-(106–115) (*C*), and E6-(71–80) (*D*). After two cycles of restimulation, CTL activity was determined in a 4-h ^{51}Cr release assay using RMA-S target cells pulse-labeled with the indicated peptides. (*A*) Targets: ●, RMA-S cells plus E6-(41–50); ○, RMA-S cells plus E6-(106–115). (*B*) Targets: ●, RMA-S cells plus E6-(81–90); ○, RMA-S cells plus E6-(41–50). (*C*) Targets: ●, RMA-S cells plus E6-(106–115); ○, RMA-S cells plus E6-(41–50). (*D*) Targets: ●, RMA-S cells plus E6-(71–80); ○, RMA-S cells plus E6-(41–50).

H-2K^b restricted. This was confirmed by the lysis of the K^b-transfected H-2^d-derived mastocytoma cell line PIHTR and the lack of lysis of control transfected PIHTR cells (Fig. 3*B*).

We have also determined CD8 and TCR expression in the anti-E6-(41–50) CTL line. Staining after 3 months in culture showed that 100% of the cells were CD8, Vβ8.1-positive (data not shown). This suggested that the T-cell line had developed into a monoclonal or oligoclonal cell population.

Since naturally processed class I-bound peptides were shown to be 8–9 amino acids long (2, 13), we have tested whether CTLs generated against the 10-mer E6-(41–50) can recognize shorter versions of this peptide. A 9-mer, E6-(42–50), was synthesized that lacked 1 amino acid at the N terminus but still contained the K^b binding anchor residue (14) at the C terminus. The peptide binding assay confirmed that the 9-mer bound to K^b (data not shown). CTLs generated against the 10-mer E6-(41–50) were able to lyse target cells pulse-labeled with 9-mer, but this required ≈20-fold higher peptide concentration (Fig. 3*C*). In contrast, CTLs generated against the 9-mer E6-(42–50) showed the opposite sensitivity pattern, recognizing 9-mer pulse-labeled targets more efficiently than targets pulsed with 10-mer (Fig. 3*D*).

In vitro-induced CTLs are not always specific for the main peptide present in the immunizing preparation (15). Thus, if 10-mer- and 9-mer-induced CTLs were specific for different by-products of the crude peptide preparations, this might explain the observed difference in the sensitivity of the effector CTLs. This possibility was excluded by experiments with HPLC-purified peptides, which showed that 10-mer- and 9-mer-containing fractions were recognized most efficiently by the respective CTLs (data not shown).

DISCUSSION

Twenty of 99 HPV16-derived peptides were found to bind to H-2K^b and/or D^b molecules expressed by mutant RMA-S cells. HPLC-purified preparations showed that class I bind-

Table 2. Ability of HPV-derived peptides to induce specific CTL responses *in vitro*

Type	Peptide	% specific lysis 12:1	% specific lysis 6:1
MHC class I binding	L1-(61–75)*	52	32
	L1-(71–85)	25	13
	L1-(91–105)*	76	51
	L1-(141–155)	25	17
	L1-(161–175)	25	27
	L1-(201–215)*	77	48
	L1-(231–245)	4	1
	L1-(251–265)	83	68
	L1-(381–395)	36	25
	L1-(471–485)	58	30
	L1-(481–495)	1	3
	L1-(501–515)	82	60
	E6-(36–45)*	80	70
	E6-(41–50)	88	77
	E6-(81–90)	50	36
	E6-(106–115)	17	15
	E7-(21–30)	42	32
	E7-(61–70)	1	0
	E7-(71–80)	39	36
	E7-(76–85)	0	3
Nonbinding control	E6-(16–25)	15	10
	E6-(56–65)	12	4
	E6-(66–75)	10	5
	E6-(71–80)	0	0
	E6-(91–105)	9	9

RMA-S cells, preincubated overnight at 25°C, were incubated for 3 h at 31°C in 100 μM peptide, mitomycin C-treated, and incubated with syngeneic splenocytes and 6 μM peptide. After a 5-day induction, CTLs underwent two further cycles of restimulation with irradiated splenocytes plus peptide and Con A supernatant. Responder T cells were harvested and tested in a ^{51}Cr release assay against RMA-S target cells pulse-labeled either with the immunizing peptides or with irrelevant class I-binding HPV peptides (see Fig. 2). Percent specific lysis is the lysis of RMA-S cells pulse-labeled with the immunizing peptides. Underlined values indicate positive peptide-specific lysis (>25%). In all assays, lysis of RMA-S cells pulse-labeled with irrelevant peptides was <10% at the effector/target cell ratios shown (12:1 and 6:1).

*Lysis was determined at effector/target cell ratios of 3:1 and 1.5:1.

ing was mediated by 15-mers and 10-mers and not by shorter peptide byproducts. These long peptides are expected to bind less efficiently than 8- and 9-mer peptides shown in recent studies to bind most efficiently to class I molecules (16, 17). For some, but not all, HPV peptides we found that 9-mer versions bound indeed more strongly to class I than the longer peptides (data not shown).

Microsequencing of H-2K^b- and D^b-eluted peptides has revealed conserved anchor amino acid residues (14). The E6, E7, and L1 proteins do not contain any D^b anchor motif sequences, but four K^b anchor motifs are present. One of these motifs was disrupted in the peptide library because it fell into a region that was not covered by the peptide overlap. All three peptides that contained intact K^b anchor motifs gave strong positive results in the class I binding assay, indicating that these motifs have useful predictive value. However, 13 class I binding peptides did not contain binding motif sequences (Table 3) and would not, therefore, have been predicted. Seven of these peptides mediated only a marginal increase of class I expression (Table 1), suggesting weak binding. However, other motif-negative peptides bound as efficiently as motif-containing peptides. We have not yet detected common sequences among the motif-negative peptides. Experiments to determine their optimal binding length and amino acid composition will provide information about

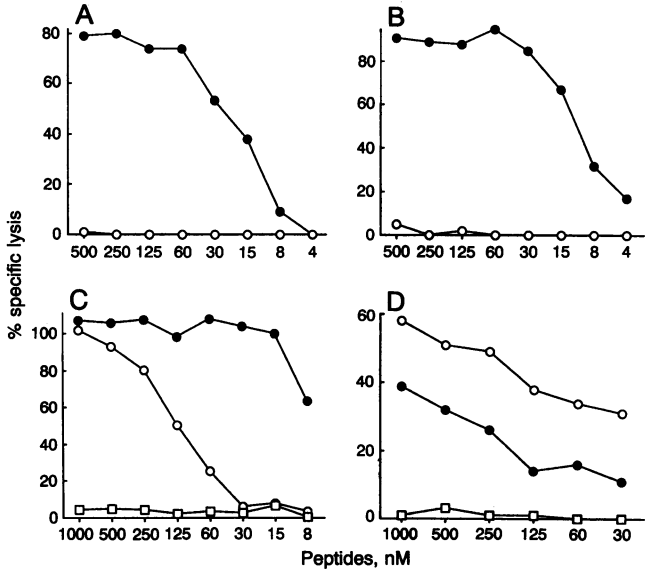

FIG. 3. Dose–response curves of peptide-specific CTLs. Long-term CTL lines induced by the 10-mer E6 peptide E6-(41–50) (*A–C*) or the 9-mer E6 peptide E6-(42–50) (*D*) were tested in a 4-h ^{51}Cr release assay against RMA-S (*A*, *C*, and *D*) targets pulse-labeled with decreasing concentrations of E6-(41–50), E6-(42–50), or irrelevant E6 peptides. In *B*, P1HTR cells transfected with H-2K^b or HLA-A2 were used as targets. (*A*) Targets: ●, RMA-S cells plus E6-(41–50); ○, RMA-S cells plus E6-(106–115). (*B*) Targets: ●, P1HTR-Kb cells plus E6-(41–50); ○, P1HTR-A2 cells plus E6-(41–50). (*C*) Targets: ●, RMA-S cells plus E6-(41–50); ○, RMA-S cells plus E6-(42–50); □, RMA-S cells plus E6-(101–110). (*D*) Targets: ○, RMA-S cells plus E6-(42–50); ●, RMA-S cells plus E6-(41–50); □, RMA-S cells plus L1-(256–264).

the interaction of these peptides with MHC class I molecules. They may be equivalent to recently described peptides that were found to be naturally presented by K^b although no anchor motif residues were detectable (18). It is therefore likely that not all K^b-presented peptides contain anchor motif sequences, suggesting limitations of a motif-based prediction of possible CTL epitopes.

In this study the majority of class I binding peptides induced CTL responses *in vitro*, whereas peptides that were negative in the binding assay did not. Only 5 of the 20 class I binding peptides failed to induce CTLs, which might be due to weak binding, as suggested for 3 of the 5 peptides by the results of the binding assay (Table 1). The remaining two peptides, however, gave strong positive results in the binding assay. The fact that one of them, E6-(106–115), efficiently stimulated CD4$^+$ but not CD8$^+$ T cells suggests that this peptide can bind to MHC class II molecules in addition to the demonstrated class I binding. The other peptide L1-(231–245) contains a K^b anchor motif. One reason for failure of this strongly K^b binding peptide to induce CTL might be that the anchor motif residues were located in the middle of a synthetic 15-mer and not at the C terminus where they are normally found. To test this a 9-mer, L1-(231–239), containing the anchor residues at the correct relative positions was synthesized and tested for class I binding and CTL induction. Although this peptide bound strongly to K^b, it did not induce specific CTL responses, suggesting that a hole in the T-cell repertoire of C57BL/10 mice is responsible for unresponsiveness. Therefore, despite the presence of an anchor motif, L1-(231–239) is unlikely to represent a natural CTL epitope in these mice.

It seems likely that natural antigen processing will limit the number of MHC class I-associated peptides and perhaps only some of the K^b and D^b binding peptides identified here will be presented in transfected cells. In this context it is impor-

Table 3. Amino acid sequences of H-2^b class I binding peptides and CTL-inducing properties

Peptide		Binding assay		CTL	Binding motif
		K^b	D^b		
L1-(61–75)	YHAGTSRLLAVGHPY	+	+	+	None
L1-(71–85)	VGHPYFPIKKPNNNK	+	−	+	K^b weak
L1-(91–105)	VSGLQYRVFRIHLPD	+	−	+	None
L1-(141–155)	VGISGHPLINKLDDT	+	+	+	None
L1-(161–175)	YAANAGVDNRECISM	+	+	+	D^b strong
L1-(201–215)	CTNVAVNPGDCPPLE	−	+	+	None
L1-(231–245)	FGAMDFTTLQANKSE	+	+	−	K^b anchor
L1-(251–265)	CTSICKYPDYIKMVS	+	+	+	K^b strong
L1-(381–395)	YKNTNFKEYLRHGEE	+	+	+	None
L1-(471–485)	TFWEYNLKEKFSADL	+	−	+	K^b weak
L1-(481–495)	FSADLDQFPLGRKFL	+	+	−	None
L1-(501–515)	KAKPKFTLGKRKATP	+	−	+	None
E6-(36–45)	QLLRREVYDF	+	−	+	None
E6-(41–50)	EVYDFAFRDL	+	−	+	K^b anchor
E6-(81–90)	YSLYGTTLEQ	+	−	+	None
E6-(106–115)	CQKPLCPEEK	+	−	−	None
E7-(21–30)	DLYCYEQLND	+	−	+	K^b anchor
E7-(61–70)	CDSTLRLCVQ	+	−	−	None
E7-(71–80)	STHVDIRTLE	+	−	+	None
E7-(76–85)	IRTLEDLLMG	+	−	−	None
SV-(323–331)	FAPGNYPAL	+	+	+	K^b anchor
NP-(365–380)	IASNENMETMESSTLE	−	+	+	D^b anchor
NP-(345–360)	SFIRGTKVSPRGKLST	+	−	+	None

The presence of anchor or strong allele-specific motifs [as described by Falk *et al.* (14)] is indicated in the last column; putative anchor residues are underlined in the sequences.

tant to notice that CTLs induced against 15- and 10-mers may not efficiently recognize naturally presented 9- and 8-mers. For example, we found that CTLs induced against the 10-mer E6-(41–50) recognized the 9-mer E6-(42–50) less efficiently than the peptide used for immunization. CTLs induced against the 9-mer showed the opposite sensitivity pattern (Fig. 3). It is likely that peptide length can affect the conformation of the peptide–MHC complex seen by the T-cell receptor. This is supported by the observation that a long-term CTL line against the 10-mer was 100% Vβ8.1-positive, whereas all CTLs of an anti-9-mer line were Vβ8.3-positive (data not shown).

The demonstrated importance of peptide length for CTL induction and the recent finding that peptides naturally presented by class I molecules were 8 or 9 amino acids long (2, 13) have critical implications for the development of peptide-based vaccines. If vaccination is designed to circumvent the need for intracellular antigen processing, it will be important to match the lengths of the immunizing and the target peptides.

We thank Drs. P. Beverley, M. Crumpton, and L. Crawford for constant support and Dr. R. Zamoyska for reading the manuscript.

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