Characterization of Two Distinct Major Histocompatibility Complex Class I K^k -Restricted T-Cell Epitopes within the Influenza A/PR/8/34 Virus Hemagglutinin

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Cytotoxic T-lymphocyte (CTL) clones specific for the influenza A/PR/8/34 virus hemagglutinin (HA) were isolated by priming CBA mice with a recombinant vaccinia virus expressing the HA molecule. The epitopes recognized by two of these clones, which were CD8⁺, K^k restricted, and HA subtype specific, were defined by using a combination of recombinant vaccinia viruses expressing HA fragments and synthetic peptides. One epitope is in the HA1 subunit at residues 259 to 266 (numbering from the initiator methionine), amino acid sequence FEANGNLI, and the other epitope is in the HA2 subunit at residues 10 to 18 (numbering from the amino terminus of the HA2 subunit), sequence IEGGWTGMI. These two peptides are good candidates for naturally processed HA epitopes presented during influenza infection, as they are the same length (eight and nine residues) as other naturally processed viral peptides presented to CTL. A comparison of the sequences of these two new epitopes with those of the three previously published K^k -restricted T-cell epitopes showed some homology among all of the epitopes, suggesting a binding motif. In particular, an isoleucine residue at the carboxy-terminal end is present in all of the epitopes. On the basis of this homology, we predicted that the K^k -restricted epitope in influenza virus nucleoprotein, previously defined as residues 50 to 63, was contained within residues 50 to 57, sequence SDYEGRLI. This shorter peptide was found to sensitize target cells at a 200-fold lower concentration than did nucleoprotein residues 50 to 63 when tested with a CTL clone, confirming the alignment of K^k -restricted epitopes.

Major histocompatibility complex (MHC) molecules bind and present immunogenic peptides to T lymphocytes as part of the immune response to viral infection (54). Peptides derived from proteins synthesized within the infected cell assemble with specific class I MHC molecules and β_2 -microglobulin and are presented to CD8⁺ cytotoxic T lymphocytes (CTL) (29, 44). This process can be mimicked in vitro by adding the appropriate T cells, target cells, and synthetic peptides to the medium (26, 47). In vivo class I molecules probably bind peptides in the endoplasmic reticulum or in a closely related intracellular compartment (30, 53). Details of the presumed proteolytic pathway and the peptide transporter required for this process are unknown, although recently a putative peptide transporter has been identified (12, 15, 28, 39, 40, 48).

Infection of mice with influenza A viruses elicits a vigorous class I MHC-restricted CTL response (52). Influenza A virus hemagglutinin (HA) is known to serve as a target antigen for influenza virus-specific murine CTL, and all three human influenza virus HA subtypes (H1, H2, and H3) are recognized by murine CTL (3, 5, 17, 46). Although there is detailed information about CTL epitopes within the influenza A/JAPAN/305/57 virus HA (H2 subtype) (7, 41, 42), only one MHC class I-restricted CTL epitope has been defined in an H1 subtype HA (A/PR/8/34) (23), and there is no information about CTL epitopes within any H3 subtype HA. The mature HA molecule consists of a heavy-chain subunit (HA1) of about 330 amino acid residues and a light-chain subunit (HA2) of about 220 amino acid residues held together by a single disulfide bond (51). We have been

studying the CTL recognition of the influenza A/PR/8/34 virus HA and have previously shown the existence of $H-2^k$ -restricted epitopes in both the HA1 and HA2 subunits of the molecule (16).

There is only minimal detailed information about epitopes recognized by $H-2^k$ MHC class I-restricted CTL. Only five such epitopes have been defined with synthetic peptides; three of these epitopes are known to be restricted by the K^k MHC molecule (1, 31, 42), one is known to be restricted by the D^k molecule (38), and the restriction element of one epitope has not been defined precisely (22). Some amino acid sequence similarity between two of the K^k -restricted epitopes has been described previously (31). In order to allow a comparison of CTL epitopes of different HA subtypes and to investigate a possible consensus sequence shared by K^k -restricted peptide epitopes, we describe here the identification of two new K^k -restricted epitopes within the influenza A/PR/8/34 virus HA. A comparison of these two new epitopes with the three previously described K^k -restricted epitopes showed that all five known epitopes have some primary amino acid sequence features in common, and we propose a motif for peptide binding to the K^k class I MHC molecule.

MATERIALS AND METHODS

Mice. CBA/Ca mice ($H-2^k$) were obtained from the specific-pathogen-free mouse breeding unit, Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom. B10.A (K^kD^d), C3H-H-2⁰ (K^dD^k), and B10.D2 (K^dD^d) mice were purchased from Olac.

Influenza viruses. Influenza viruses A/PR/8/34 (Mount Sinai strain, H1 subtype), A/JAPAN/305/57 (H2 subtype),

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and A/NT/60/68 (H3 subtype) were grown in the allantoic sacs of 11-day-old embryonated chicken eggs.

Synthetic peptides. Pin peptides were made by using an Epitope Mapping Kit (Cambridge Research Biochemicals Ltd., Cambridge, United Kingdom) according to the manufacturer's instructions. All peptides were synthesized with an additional dipeptide sequence (Asp-Pro) at the carboxy-terminal end to allow cleavage from the pins (50). Cleavage was done in capped glass vials at 39°C for 23 h by using 1 ml of 70% formic acid, and the samples were then lyophilized. Each peptide was dissolved in 0.2 ml of phosphate-buffered saline (PBS) and stored at 4°C. These pin peptides were used without further purification. Additional peptides were synthesized on a peptide synthesizer (model 430A, Applied Biosystems Inc.) by using standard methods. Deprotected peptides were dissolved in 0.1 M ammonium bicarbonate and purified on a C-18 or C-8 reverse phase high-performance liquid chromatography (HPLC) column with gradients of acetonitrile in 10 mM ammonium acetate.

Recombinant vaccinia viruses. Recombinant vaccinia viruses expressing fragments of the influenza A/PR/8/34 virus (Mount Sinai) HA (8) were made by using standard procedures (24) by insertion into the vaccinia virus *tk* gene with new shuttle vectors whose cloning sites are shown in Fig. 3. The vectors are all derived from plasmid pGS62 (11) and use the vaccinia virus 7,500-molecular-weight (7.5K) promoter for expression. The fragments of the HA cloned in the vaccinia virus vectors and their nomenclature are shown diagrammatically in Fig. 2. H1-VAC (16) and L⁻H1-VAC (45) have been described previously.

HA-specific CTL clones. Three-month-old female CBA mice were primed intravenously by injecting 10⁷ PFU of H1-VAC recombinant vaccinia virus in 0.1 ml of PBS into their tail veins. Two weeks later, their spleens were removed and the spleen cells were stimulated in vitro with influenza A/PR/8/34 virus-infected, 2,000-rad irradiated feeder spleen cells (46). The effector spleen cells were subsequently restimulated with antigen at weekly intervals in the same way, and after 3 weeks of culture in vitro the supernatant from phorbol myristate acetate-stimulated EL4 cells was added to the medium as a source of growth factors. The polyclonal CTL line established in this way was tested in a ⁵¹Cr-release assay to ensure that HA-specific CTL were present. After 8 weeks of culture in vitro, the CTL were cloned by limiting dilution to 0.5 cells per well in 96-well dishes. Actively growing clones were expanded into 24-well dishes and later into 25-cm² flasks. After clones had become well established, human recombinant interleukin 2 (rIL-2) (Cetus Corp.) was used as the growth factor at a concentration of 10 U/ml. Subsequently, the CTL clones were found to grow optimally in a mixture of rIL-2 (10 U/ml) and EL4 supernatant (2% vol). Once the peptide epitope recognized by the clones became known, the cultures were maintained on feeder cells pulsed with 0.1 mM peptide (1). Staining with fluorescein isothiocyanate-conjugated anti-CD8 and anti-CD4 antibodies (Sera-Lab) was used to distinguish these T-cell markers. The MHC restriction element used by the clones was determined by using peritoneal exudate macrophages derived from B10.A (*K^dD^d*), C3H-H-2⁰ (*K^dD^d*), and B10.D2 (*K^dD^d*) mice as target cells. Macrophages were induced by injecting 1 ml of 3% (wt/vol) thioglycolate intraperitoneally 4 days prior to harvesting.

CTL specific for influenza virus nucleoprotein residues 50 to 63. CBA mice were primed with 100 hemagglutinin units of influenza A/PR/8/34 virus and an effector cell line established in vitro, as described above, by using the same growth

factors. After five stimulations with A/PR/8/34-infected feeder cells, subsequent restimulations used spleen cells pulsed with peptide corresponding to influenza virus nucleoprotein residues 50 to 63, a known *K^k*-restricted T-cell epitope (1). The resulting CTL were cloned by limiting dilution, as described above for the HA-specific CTL. One CTL clone, NP3, was used in experiments.

Cytotoxic assay. A standard 5-h ⁵¹Cr-release assay was used to measure cytotoxicity with modifications for the use of adherent L929 target cells (*H-2^k*), as described previously (46). Target cells (4 × 10⁶) were infected with 10 PFU of vaccinia virus or influenza virus per cell as infectious allantoic fluid or left uninfected and labeled with 0.3 mCi of ⁵¹Cr for 90 min at 37°C. After two washes, target cells were left at 37°C for 2 h to express antigen, washed two more times, and plated out in the assay. In experiments with synthetic peptides, fresh stock solutions of peptide (1 mg/ml) in assay medium were made and appropriate dilutions were used. Peptides were present throughout the assay. The percentage of specific ⁵¹Cr release was calculated as follows: percent release = 100 × (release by CTL – medium release)/(2.5% Triton release – medium release). Each point was measured in duplicate against quadruplicate controls. Spontaneous release was between 13 and 29% in all experiments and was not affected by any of the synthetic peptides used.

RESULTS

Initial characterization of CTL clones. Priming CBA mice with a recombinant vaccinia virus containing a full-length cDNA of the influenza A/PR/8/34 virus HA (H1-VAC) led to the production of eight T-cell clones (see Materials and Methods), two of which, HA8 and HA11, were chosen for further study because they had growth rates more vigorous than the rest. In ⁵¹Cr-release assays, both clones lysed cells infected with influenza A/PR/8/34 virus, H1-VAC, or a recombinant vaccinia virus expressing a leader minus version of the HA (L⁻H1-VAC) (45), whereas uninfected or wild-type vaccinia virus (WR-VAC)-infected cells were not lysed (Fig. 1). The two CTL clones are clearly HA specific. However, there were clear differences in the efficiency of lysis of the different targets. Cells infected with L⁻H1-VAC or influenza A/PR/8/34 virus were lysed most efficiently, whereas cells infected with H1-VAC were relatively poor targets. There was also a difference between HA8 and HA11 in their efficiencies of lysis of H1-VAC-infected cells, clone HA8 being less efficient (Fig. 1). This suggested that the two clones were different.

Both CTL clones are CD8⁺ and are restricted by the *K^k* class I MHC molecule, as expected (2) (see Materials and Methods; results not shown). Both clones are HA subtype specific and did not lyse cells infected with influenza A/JAPAN/305/57 virus (H2 subtype) or A/NT/60/68 virus (H3 subtype) (results not shown).

Characterization of clones HA8 and HA11 by using recombinant vaccinia viruses expressing HA fragments. In order to locate the part(s) of the HA molecule recognized by CTL clones HA8 and HA11, various deletion mutants of the A/PR/8/34 HA were constructed (Fig. 2) and expressed as recombinant vaccinia viruses. In order to allow straightforward subcloning of the HA gene restriction fragments, new shuttle plasmids for the production of the recombinant vaccinia viruses were constructed (Fig. 3a). The plasmids were all derived from pGS62 (11) and use the vaccinia virus 7.5K promoter for expression. One of the plasmids, pKG33, was designed for the cloning and expression of any DNA

FIG. 1. Characterization of CTL clones. A ⁵¹Cr-release assay was carried out as described in Materials and Methods by using clones HA8 and HA11 as effector CTL and L929 target cells infected with L⁻H1-VAC (▲), influenza A/PR/8/34 virus (□), H1-VAC (○), or WR-VAC (Δ) or uninfected (●).

fragment with blunt ends. This plasmid contains an ATG initiation codon within the consensus ribosome recognition sequence (21), followed by three blunt-end restriction enzyme sites, one in each reading frame, and three TGA termination codons, one in each reading frame (Fig. 3a). Proteins expressed with this plasmid are fusion proteins, with a few extra amino acids at each end. KG34-VAC and KG35-VAC were constructed with this shuttle vector and have nine and eight extra amino acids, respectively, not derived from the HA sequence.

When the eight different recombinant viruses were used to infect L929 target cells and tested for recognition by CTL clones HA8 and HA11, a clear pattern of lysis was observed

(Fig. 4). From these results, it was clear that the epitope recognized by clone HA8 is at the amino-terminal end of the HA2 subunit, between amino acids 344 and 413 (numbering from the initiator methionine), because only KG26- and KG34-VAC-infected cells were recognized. The epitope recognized by clone HA11 is towards the carboxy-terminal end of the HA1 subunit, between amino acids 228 and 289, because KG24-, KG25-, KG26-, and KG35-VAC-infected cells were recognized (Fig. 4).

Definition of the epitopes recognized by HA8 and HA11 by using synthetic peptides. In order to identify precisely the epitopes recognized by the CTL clones, short peptides were chemically synthesized and tested for their abilities to sen-

FIG. 2. The design of recombinant vaccinia viruses expressing HA fragments. The restriction enzyme sites shown are unique within the A/PR/8/34 HA gene, except for the two PvuII sites (8). The StuI site was made by oligonucleotide-directed mutagenesis (16). The numbering shows the amino acid residue number, beginning at the initiator methionine. The regions of the HA recognized by CTL clones HA8 and HA11 are also shown.

FIG. 3. Construction of recombinant vaccinia viruses expressing HA fragments. (a) Restriction enzyme cloning sites of the new shuttle vectors used. (b) HA restriction enzyme fragment and vector used for the construction of each recombinant vaccinia virus.

sitize target cells in ^{51}Cr -release assays. For clone HA8, all 57 possible peptides that overlapped by one residue, were 12 residues long, and covered the region defined with recombinant vaccinia viruses (residues 344 to 413) were synthesized

by using pin peptide technology (see Materials and Methods). When these peptides were used in a ^{51}Cr -release assay (Fig. 5), a clear peak was observed even though the percentage of specific lysis was low. After the location of the epitope

FIG. 4. Recognition of recombinant vaccinia viruses expressing HA fragments by CTL clones HA8 and HA11. L929 cells were infected with the indicated recombinant vaccinia virus and tested as targets by using either clone HA8 (●) or clone HA11 (□) as effector cells.

FIG. 5. Recognition of pin peptides by CTL clone HA8. Overlapping pin peptides were synthesized as described in Materials and Methods. The peptides cover residues 344 to 413 of the HA molecule. L929 target cells were incubated with a quarter of the total synthesis of each peptide in a final volume of 0.15 ml, with clone HA8 added at an effector/target ratio of 5:1.

recognized by clone HA8 was defined with an impure pin peptide, the same 12-residue-long peptide, IEGGWTGMIDGW, and three shorter peptides derived from this sequence were synthesized on a larger scale and purified by reverse-phase HPLC. These peptides were then used at concentrations ranging from 10^{-4} to 10^{-10} M in ^{51}Cr -release assays with L929 target cells and clone HA8 (Fig. 6). The peptide which gave efficient lysis at the lowest concentration was nine residues long, had the sequence IEGGWTGMI (residues 354 to 362 of the HA or 10 to 18 of the HA2 subunit), and gave half-maximal lysis at a concentration of about 4 nM. Removal of a single amino acid residue from either end of this peptide greatly decreased the efficiency of target cell sensitization (Fig. 6). In particular, removal of the carboxy-terminal isoleucine residue led to a complete loss of recognition by clone HA8 over the peptide concentration range tested.

To characterize the precise epitope recognized by clone HA11, information about the K^k-restricted epitope at residues 255 to 271 in the HA of influenza A/JAPAN/305/57 virus (42) was used in conjunction with the preliminary location (see above) in designing peptides to be synthesized and tested. The epitope in the A/JAPAN/305/57 HA is within the equivalent region of the A/PR/8/34 HA recognized by clone HA11 as defined with recombinant vaccinia viruses (see above). Therefore, the corresponding 17-residue-long peptide from the A/PR/8/34 sequence, FEANGNLIAPMYA FALS, differing by six residues from the A/JAPAN/305/57 HA sequence, was synthesized, purified, and tested in a ^{51}Cr -release assay. Because this peptide was difficult to dissolve in tissue culture medium, it was dissolved in dimethylsulfoxide, and at the highest final concentration used in the assay it was diluted 300-fold in tissue culture medium. The final concentration of dimethylsulfoxide had no effect in the assay. Clone HA11 was able to lyse L929 cells incubated with this peptide, with half-maximal lysis at a peptide concentration of about 40 nM (Fig. 7). Four shorter peptides derived from this 17-residue-long sequence were also made,

FIG. 6. The effect of peptide concentration on the recognition of purified synthetic peptides by clone HA8. L929 target cells and clone HA8 effector cells were incubated at an effector/target ratio of 5:1 in the presence of synthetic peptides at the indicated concentrations in a chromium-release assay.

FIG. 7. The effect of peptide concentration on the recognition of purified synthetic peptides by clone HA11. L929 target cells and clone HA11 effector cells were incubated at an effector/target ratio of 5:1 in the presence of synthetic peptides at the indicated concentrations in a chromium-release assay.

TABLE 1. Alignment of known K^k -restricted T-cell epitopes

Sequence ^a	MHC	Reference
SD - Y E G R L I	K^k	1
FE - S T G N L I	K^k	42
FE - A N G N L I	K^k	This paper
I E G G W T G M I	K^k	This paper
R S E F L L E K R I	K^k	31
Q N S L T I		
A P E Y G F K I S		
I Q S G I A L L		
C K M E K C S	$H-2^k$	22
E K K I C K M E K C S	$H-2^k$	22

^a The last two sequences show two possible alignments of an $H-2^k$ -restricted epitope of the circumsporozoite protein of *Plasmodium falciparum* whose restriction element has not been precisely defined.

purified, and used over a range of concentrations in a ^{51}Cr -release assay with clone HA11. The peptide which gave efficient lysis at the lowest concentration was eight residues long, had the sequence FEANGNLI (residues 259 to 266 of the HA1 subunit), and gave half-maximal lysis at a concentration of about 1 nM (Fig. 7). Removal of a single amino acid residue from either end of this peptide greatly decreased the efficiency of target cell sensitization (Fig. 7).

The K^k -restricted CTL epitope in influenza virus nucleoprotein. When the amino acid sequences of the two new CTL epitopes within the A/PR/8/34 HA identified in this report were compared with the sequences of the three previously described K^k -restricted T-cell epitopes (1, 31, 42), all five sequences showed some homology (Table 1). The optimum-length peptides for the previously published epitopes have not been described, but they are predicted by the alignment shown in Table 1. The prediction for one of these epitopes, previously defined as influenza virus nucleoprotein residues 50 to 63 (1), was tested by synthesizing the shorter peptide, SDYEGRLI (residues 50 to 57), and comparing the efficiencies of target cell sensitization by the two peptides by using a CTL clone specific for this epitope (Fig. 8). As predicted, the shorter peptide, residues 50 to 57, gave efficient lysis at much lower concentrations than peptide residues 50 to 63 (Fig. 8).

DISCUSSION

In previous work, we have shown the existence of $H-2^k$ -restricted T-cell epitopes in both the HA1 and HA2 subunits of the influenza A/PR/8/34 virus HA molecule (16). In this report, we have identified these epitopes precisely by using HA-specific CTL clones and a combination of recombinant vaccinia viruses and synthetic peptides. The HA1 epitope (eight residues long) corresponds to residues 259 to 266, and the HA2 epitope (nine residues long) corresponds to residues 354 to 362. The epitope within the HA2 subunit recognized by CTL clone HA8 is 10 residues C terminal to the junction between the HA1 and HA2 subunits, a known proteolytic cleavage site. A trypsinlike protease is responsible for cleavage at the arginine residue at this site in influenza virus-infected cells (19). Because processing is required for the presentation of antigens to MHC class I-restricted CTL (44), it is possible that this same arginine residue is used in antigen processing of the HA in vivo.

Braciale and coworkers have defined four CTL epitopes within the influenza A/JAPAN/305/57 virus HA (H2 subtype), one K^k -restricted epitope (42) and three K^d -restricted epitopes (7, 41). In the case of the K^d -restricted epitope

within the transmembrane region of the molecule (6), it has been shown that there is an equivalent epitope in the A/PR/8/34 HA (23), and CTL that recognize this epitope may be cross-reactive between the two HA subtypes. In this paper, we show that the K^k -restricted epitopes in the HA1 subunits of A/PR/8/34 and A/JAPAN/305/57 are also conserved in homologous positions. However, the characterized CTL specific for these epitopes are not cross-reactive between the two HA subtypes, even though there are only two amino acid differences between the two epitopes (Table 1). This suggests that one or both of these adjacent amino acids are important T-cell receptor contact residues. By contrast, there is no known epitope in the A/JAPAN/305/57 HA equivalent to the K^k -restricted epitope in the HA2 subunit of the A/PR/8/34 HA.

It has been shown previously that in vaccinia virus-infected cells there is a defect in the presentation of A/PR/8/34 HA epitopes to MHC class I-restricted CTL (10) which

FIG. 8. The effect of peptide concentration on the recognition of purified synthetic peptides by clone NP3, a CTL clone specific for the K^k -restricted epitope of influenza virus nucleoprotein (see Materials and Methods). L929 target cells and clone NP3 effector cells were incubated at an effector/target ratio of 5:1 in the presence of synthetic peptides at the indicated concentrations in a chromium-release assay.

can be overcome by redirecting HA into the cytoplasm (43). This was confirmed and extended in the present study by using HA-specific CTL clones (Fig. 1). The effect of vaccinia virus infection is probably due to an inhibition of proteolytic processing by vaccinia virus-encoded protease inhibitors (4, 20, 37). There was a difference in the extent of the vaccinia virus block for the two HA epitopes (Fig. 1, H1-VAC recognition by clones HA8 and HA11), suggesting that the processing required to generate the two epitopes may be different. The inhibition of HA epitope presentation by vaccinia virus infection could be overcome most effectively by an amino-terminal leader minus HA of cytoplasmic location (L⁻H1-VAC; Fig. 1), as reported previously with polyclonal T cells (43).

Several methods have been proposed for predicting the location of T-cell epitopes within primary amino acid sequences (9, 25, 32, 33). Because of the high degree of polymorphism between MHC molecules, it seems intrinsically unlikely that any one method will be able to predict all T-cell epitopes. However, for epitopes presented by one particular MHC molecule, it is possible that a consensus sequence for epitope binding may exist. In agreement with this hypothesis, characterization of self-peptides eluted from different MHC class I molecules has provided good evidence of allele-specific peptide binding motifs (13), although no information about self-peptides eluted from the K^k molecule was reported. A comparison of the two new K^k-restricted A/PR/8/34 HA epitopes presented in this report with the three previously described K^k-restricted CTL epitopes shows that they share some common features (Table 1). They all have a glutamic or aspartic acid residue one residue from the amino terminus at position 2 and an isoleucine residue at the carboxy terminus, and we propose that these two residues form the motif for binding to the K^k molecule. There is a close correlation of this motif with the derived motifs for binding to four other class I molecules (13); all of these motifs have two principal anchor residues, including one hydrophobic residue at the carboxy terminus. The length of the optimal HA peptide epitopes presented by the K^k class I molecule, eight and nine residues, is the same as the length of both self-peptides and viral peptides eluted from class I MHC molecules (13, 34, 49). Class I MHC molecules have been shown to selectively bind peptides of this length, even when present as a minor component in a mixture of longer peptides (35). The A/PR/8/34 HA peptide epitopes described in this report are therefore good candidates for naturally processed epitopes presented during influenza virus infection. The optimal peptides for CTL recognition have not been described for all known K^k-restricted epitopes, but they are predicted by the alignment shown in Table 1. One of these predictions, for the epitope previously defined as influenza virus nucleoprotein residues 50 to 63, was tested and confirmed (Fig. 8), providing evidence that the alignment is correct. On the basis of this alignment of epitopes, we also predict that the malarial H-2K^b-restricted epitope whose restriction element has not been defined precisely (22) is also K^k restricted (Table 1).

Detailed studies on other defined T-cell epitopes have shown that relatively few amino acid residues are important for binding to MHC molecules (14, 18, 27, 36). In the case of one particular K^d-restricted epitope, a tyrosine residue adjacent to the amino terminus was most critical for epitope binding (27). A tyrosine residue was also important for MHC binding by three other different K^d-restricted epitopes and has been proposed to be a general feature of epitopes which are presented by the K^d molecule (27). In addition, tyrosine

in position 2 was found to be an anchor residue for self-peptides eluted from the K^d molecule (13). The proposed motif for binding to the K^k molecule is similar to that for the K^d molecule, with a glutamic or aspartic acid residue instead of tyrosine at position 2 and isoleucine or leucine at the carboxy terminus. Computer modeling studies to try to identify suitable binding pockets within the MHC molecules for these consensus features are currently in progress.

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REFERENCES

1. Bastin, J., J. Rothbard, J. Davey, I. Jones, and A. Townsend. 1987. Use of synthetic peptides of influenza nucleoprotein to define epitopes recognized by class I-restricted cytotoxic T lymphocytes. *J. Exp. Med.* 165:1508-1523.
2. Bennink, J. R., and J. W. Yewdell. 1988. Murine cytotoxic T lymphocyte recognition of individual influenza virus proteins—high frequency of nonresponder MHC class I alleles. *J. Exp. Med.* 168:1935-1939.
3. Bennink, J. R., J. W. Yewdell, G. L. Smith, and B. Moss. 1986. Recognition of cloned influenza virus hemagglutinin gene products by cytotoxic T lymphocytes. *J. Virol.* 57:786-791.
4. Boursnell, M. E. G., I. J. Foulds, J. I. Campbell, and M. M. Binns. 1988. Non-essential genes in the vaccinia virus HindIII K fragment: a gene related to serine protease inhibitors and a gene related to the 37K vaccinia virus major envelope antigen. *J. Gen. Virol.* 69:2995-3003.
5. Braciale, T. J., V. L. Braciale, T. J. Henkel, J. Sambrook, and M.-J. Gething. 1984. Cytotoxic T lymphocyte recognition of the influenza hemagglutinin gene product expressed by DNA-mediated gene transfer. *J. Exp. Med.* 159:341-354.
6. Braciale, T. J., V. L. Braciale, M. Winkler, I. Stroynowski, L. Hood, J. Sambrook, and M.-J. Gething. 1987. On the role of the transmembrane anchor sequence of influenza hemagglutinin in target cell recognition by class I MHC-restricted, hemagglutinin-specific cytolytic T lymphocytes. *J. Exp. Med.* 166:678-692.
7. Braciale, T. J., M. T. Sweetser, L. A. Morrison, D. J. Kittleson, and V. L. Braciale. 1989. Class I major histocompatibility complex-restricted cytolytic T lymphocytes recognize a limited number of sites on the influenza hemagglutinin. *Proc. Natl. Acad. Sci. USA* 86:277-281.
8. Caton, A. J., G. G. Brownlee, J. W. Yewdell, and W. Gerhard. 1982. The antigenic structure of the influenza virus A/PR/8/34 hemagglutinin (H1 subtype). *Cell* 31:417-427.
9. Claverie, J.-M., P. Kourilsky, P. Langlade-Demoyen, A. Chaloufour-Prochnicka, G. Dadaglio, F. Tekaiia, F. Plata, and L. Bougueleret. 1988. T-immunogenic peptides are constituted of rare sequence patterns. Use in the identification of T epitopes in the human immunodeficiency virus gag protein. *Eur. J. Immunol.* 18:1547-1553.
10. Coupar, B. E. H., M. E. Andrew, G. W. Both, and D. B. Boyle. 1986. Temporal regulation of influenza hemagglutinin expression in vaccinia virus recombinants and effects on the immune response. *Eur. J. Immunol.* 16:1479-1487.
11. Cranage, M. P., T. Kouzarides, A. T. Bankier, S. Satchwell, K. Weston, P. Tomlinson, B. Barrell, H. Hart, S. E. Bell, A. C. Minson, and G. L. Smith. 1986. Identification of the human cytomegalovirus glycoprotein B gene and induction of neutralizing antibodies via its expression in recombinant vaccinia virus. *EMBO J.* 5:3057-3063.
12. Deverson, E. V., I. R. Gow, W. J. Coadwell, J. J. Monaco, G. W. Butcher, and J. C. Howard. 1990. MHC class II region encoding proteins related to the multidrug resistance family of transmembrane transporters. *Nature (London)* 348:738-741.
13. Falk, K., O. Rotzschke, S. Stevanovic, G. Jung, and H.-G.

- Rammensee. 1991. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature (London)* **351**:290-296.
14. Gotch, F., A. McMichael, and J. Rothbard. 1988. Recognition of influenza A matrix protein by HLA-A2-restricted cytotoxic T lymphocytes—use of analogues to orientate the matrix peptide in the HLA-A2 binding site. *J. Exp. Med.* **168**:2045-2057.
 15. Gould, K., J. Cossins, J. Bastin, G. G. Brownlee, and A. Townsend. 1989. A 15 amino acid fragment of influenza nucleoprotein synthesized in the cytoplasm is presented to class I-restricted cytotoxic T lymphocytes. *J. Exp. Med.* **170**:1051-1056.
 16. Gould, K. G., H. Scotney, A. R. M. Townsend, J. Bastin, and G. G. Brownlee. 1987. Mouse H-2^k-restricted cytotoxic T cells recognize antigenic determinants in both the HA1 and HA2 subunits of the influenza A/PR/8/34 haemagglutinin. *J. Exp. Med.* **166**:693-701.
 17. Itamura, S., H. Iinuma, H. Shida, Y. Morikawa, K. Nerome, and A. Oya. 1990. Characterization of antibody and cytotoxic T lymphocyte responses to human influenza virus H3 haemagglutinin expressed from the haemagglutinin locus of vaccinia virus. *J. Gen. Virol.* **71**:2859-2865.
 18. Jardetzky, T. S., J. C. Gorga, R. Busch, J. Rothbard, J. L. Strominger, and D. C. Wiley. 1990. Peptide binding to HLA-DR1: a peptide with most residues substituted to alanine retains MHC binding. *EMBO J.* **9**:1797-1803.
 19. Klenk, H.-D., R. Rott, M. Orlich, and J. Blodorn. 1975. Activation of influenza A viruses by trypsin treatment. *Virology* **68**:426-439.
 20. Kotwal, G. J., and B. Moss. 1989. Vaccinia virus encodes two proteins that are structurally related to members of the plasma serine protease inhibitor superfamily. *J. Virol.* **63**:600-606.
 21. Kozak, M. 1987. An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res.* **15**:8125-8148.
 22. Kumar, S., L. H. Miller, I. A. Quakyi, D. B. Keister, R. A. Houghten, W. L. Maloy, B. Moss, J. A. Berzofsky, and M. F. Good. 1988. Cytotoxic T cells specific for the circumsporozoite protein of *Plasmodium falciparum*. *Nature (London)* **334**:258-260.
 23. Kuwano, K., T. J. Braciale, and F. A. Ennis. 1989. Cytotoxic T lymphocytes recognize a cross-reactive epitope on the transmembrane region of influenza H1 and H2 haemagglutinins. *Viral Immunol.* **2**:163-173.
 24. Mackett, M., G. L. Smith, and B. Moss. 1984. General method for production and selection of infectious vaccinia virus recombinants expressing foreign genes. *J. Virol.* **49**:857-864.
 25. Margalit, H., J. L. Spouge, J. L. Cornette, K. B. Cease, C. DeLisi, and J. A. Berzofsky. 1987. Prediction of immunodominant helper T cell antigenic sites from the primary sequence. *J. Immunol.* **138**:2213-2229.
 26. Maryanski, J. L., P. Pala, G. Corradin, B. R. Jordan, and J.-C. Cerottini. 1986. H-2-restricted cytolytic T cells specific for HLA can recognize a synthetic HLA peptide. *Nature (London)* **324**:578-579.
 27. Maryanski, J. L., A. S. Verdini, P. C. Weber, F. R. Salemme, and G. Corradin. 1990. Competitor analogs for defined T cell antigens: peptides incorporating a putative binding motif and polyproline or polyglycine spacers. *Cell* **60**:63-72.
 28. Monaco, J. J., S. Cho, and M. Attaya. 1990. Transport protein genes in the murine MHC: possible implications for antigen processing. *Science* **250**:1723-1726.
 29. Morrison, L. A., A. E. Lukacher, V. L. Braciale, D. P. Fan, and T. J. Braciale. 1986. Differences in antigen presentation to MHC class I- and class II-restricted influenza virus-specific cytolytic T lymphocyte clones. *J. Exp. Med.* **163**:903-921.
 30. Nuchtern, J. G., J. S. Bonifacio, W. E. Biddison, and R. D. Klausner. 1989. Brefeldin A implicates egress from the endoplasmic reticulum in class I restricted antigen presentation. *Nature (London)* **339**:223-226.
 31. Rawle, F. C., K. A. O'Connell, R. W. Geib, B. Roberts, and L. R. Gooding. 1988. Fine mapping of an H-2K^k restricted cytotoxic T lymphocyte epitope in SV40 T antigen by using in-frame deletion mutants and a synthetic peptide. *J. Immunol.* **141**:2734-2739.
 32. Reyes, V. E., L. T. Chin, and R. E. Humphreys. 1988. Selection of class I MHC-restricted peptides with the strip-of-helix hydrophobicity algorithm. *Mol. Immunol.* **25**:867-871.
 33. Rothbard, J. B., and W. R. Taylor. 1988. A sequence pattern common to T cell epitopes. *EMBO J.* **7**:93-100.
 34. Rotzschke, O., K. Falk, K. Deres, H. Schild, M. Norda, J. Metzger, G. Jung, and H.-G. Rammensee. 1990. Isolation and analysis of naturally processed viral peptides as recognized by cytotoxic T cells. *Nature (London)* **348**:252-254.
 35. Schumacher, T. N. M., M. L. H. De Bruijn, L. N. Vernie, W. M. Kast, C. J. M. Melief, J. J. Neefjes, and H. L. Ploegh. 1991. Peptide selection by MHC class I molecules. *Nature (London)* **350**:703-706.
 36. Sette, A., S. Buus, S. Colon, J. A. Smith, C. Miles, and H. M. Grey. 1987. Structural characteristics of an antigen required for its interaction with Ia and recognition by T cells. *Nature (London)* **328**:395-399.
 37. Smith, G. L., S. T. Howard, and Y. S. Chan. 1989. Vaccinia virus encodes a family of genes with homology to serine proteinase inhibitors. *J. Gen. Virol.* **70**:2333-2343.
 38. Song, E. S., R. Linsk, C. A. Olson, M. McMillan, and R. S. Goodenow. 1988. Allospecific cytotoxic T lymphocytes recognize an H-2 peptide in the context of a murine major histocompatibility complex class I molecule. *Proc. Natl. Acad. Sci. USA* **85**:1927-1931.
 39. Spies, T., M. Bresnahan, S. Bahram, D. Arnold, G. Blanck, E. Mellins, D. Pious, and R. DeMars. 1990. A gene in the human major histocompatibility complex class II region controlling the class I antigen presentation pathway. *Nature (London)* **348**:744-747.
 40. Spies, T., and R. DeMars. 1991. Restored expression of major histocompatibility class I molecules by gene transfer of a putative peptide transporter. *Nature (London)* **351**:323-324.
 41. Sweetser, M. T., V. L. Braciale, and T. J. Braciale. 1989. Class I major histocompatibility complex-restricted T lymphocyte recognition of the influenza haemagglutinin. *J. Exp. Med.* **170**:1357-1368.
 42. Sweetser, M. T., L. A. Morrison, V. L. Braciale, and T. J. Braciale. 1989. Recognition of pre-processed endogenous antigen by class I but not class II MHC-restricted T cells. *Nature (London)* **342**:180-182.
 43. Townsend, A., J. Bastin, K. Gould, G. Brownlee, M. Andrew, B. Coupar, D. Boyle, S. Chan, and G. Smith. 1988. Defective presentation to class I-restricted cytotoxic T lymphocytes in vaccinia-infected cells is overcome by enhanced degradation of antigen. *J. Exp. Med.* **168**:1211-1224.
 44. Townsend, A., and H. Bodmer. 1989. Antigen recognition by class I-restricted T lymphocytes. *Annu. Rev. Immunol.* **7**:601-624.
 45. Townsend, A. R. M., J. Bastin, K. Gould, and G. G. Brownlee. 1986. Cytotoxic T lymphocytes recognize influenza haemagglutinin that lacks a signal sequence. *Nature (London)* **324**:575-577.
 46. Townsend, A. R. M., A. J. McMichael, N. P. Carter, J. A. Huddleston, and G. G. Brownlee. 1984. Cytotoxic T cell recognition of the influenza nucleoprotein and haemagglutinin expressed in transfected mouse L cells. *Cell* **39**:13-25.
 47. Townsend, A. R. M., J. Rothbard, F. M. Gotch, G. Bahadur, D. Wraith, and A. J. McMichael. 1986. The epitopes of influenza nucleoprotein recognised by cytotoxic T lymphocytes can be defined with short synthetic peptides. *Cell* **44**:959-968.
 48. Trowsdale, J., I. Hanson, I. Mockridge, S. Beck, A. Townsend, and A. Kelly. 1990. Sequences encoded in the class II region of the MHC related to the 'ABC' superfamily of transporters. *Nature (London)* **348**:741-744.
 49. Van Bleek, G. M., and S. G. Nathenson. 1990. Isolation of an endogenously processed immunodominant viral peptide from the class I H-2K^b molecule. *Nature (London)* **348**:213-216.
 50. Van der Zee, R., W. Van Eden, R. H. Melen, A. Noordzij, and

- J. D. A. Van Embden.** 1989. Efficient mapping and characterization of a T cell epitope by the simultaneous synthesis of multiple peptides. *Eur. J. Immunol.* **19**:43–47.
51. **Wiley, D. C., and J. J. Skehel.** 1987. The structure and function of the hemagglutinin membrane glycoprotein of influenza virus. *Annu. Rev. Biochem.* **56**:365–394.
52. **Wraith, D. C.** 1987. The recognition of influenza A virus-infected cells by cytotoxic T lymphocytes. *Immunol. Today* **8**:239–246.
53. **Yewdell, J. W., and J. R. Bennink.** 1989. Brefeldin A specifically inhibits presentation of protein antigens to cytotoxic T lymphocytes. *Science* **244**:1072–1075.
54. **Yewdell, J. W., and J. R. Bennink.** 1990. The binary logic of antigen processing and presentation to T cells. *Cell* **62**:203–206.