SYNTHETIC PEPTIDES AS ANTIGENS AND COMPETITORS IN RECOGNITION BY H-2-RESTRICTED CYTOLYTIC T CELLS SPECIFIC FOR HLA

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It is widely accepted that T lymphocytes recognize antigens in the context of cell surface glycoproteins encoded by genes in the major histocompatibility complex (MHC) (reviewed in references 1 and 2). In general, cytolytic T cells (CTL) recognize antigens in association with class I MHC molecules, whereas helper T cells are class II restricted. The molecular details of the presumed interaction between MHC molecules, antigen, and antigen-specific T cell receptors have not yet been elucidated. In class II–restricted systems, the characterization of antigens recognized by T cells has been simplified by the finding that T cells specific for certain protein antigens could be stimulated in the presence of appropriate MHC class II–bearing cells by well-defined protein fragments obtained either by enzymatic or chemical cleavage of the protein or by chemical synthesis (3–10). Recently, Townsend et al. (11) demonstrated that class I–restricted CTL specific for influenza virus nucleoprotein (NP)[1] could lyse uninfected target cells in the presence of synthetic NP peptides. These results suggest that a common function of class I and class II cell surface glycoproteins is to present antigenic fragments to T cells.

We have recently described a system that may be useful for studying MHC class I–restricted antigen recognition by CTL (12). We derived a series of DBA/2 (H-2^d) CTL clones directed against syngeneic P815 cells transfected with cloned human MHC class I (HLA) genes. An unexpected but interesting feature of these clones was that they apparently recognized HLA only in the context of a mouse MHC class I (H-2) restriction element. For example, CTL that lysed P815 cells transfected with HLA-CW3 failed to lyse mouse L cells (H-2^k haplotype) transfected with the same HLA gene or $CW3^+$ human cells. However, these anti-CW3 CTL could clearly lyse L cells transfected with both HLA-CW3 and H-$2K^d$ class I genes. Similar results were obtained with anti-HLA-A24 CTL. Moreover, human cells transfected with K^d were also specifically lysed (13). By comparing the pattern of lysis obtained on a panel of P815 target cells transfected with different HLA class I genes or with recombinant exon-shuffled HLA genes, we localized an antigenic epitope recognized by one of the clones to the second external (α2) domain of HLA-CW3. We subsequently found that this and

[1] *Abbreviations used in this paper:* cyt *c*, cytochrome *c*; HA, hemagglutinin; NP, nucleoprotein.

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several other anti-CW3 CTL clones could lyse (HLA^-) P815 cells incubated with synthetic peptides corresponding to residues 171–186 or 171–182 of HLA-CW3 (14–16).

In the present study, we show that antigens recognized by K^d-restricted CTL specific for HLA-A24 can also be mimicked by synthetic peptides, and that several distinct CW3 and A24 epitopes map to the COOH-terminal end of the $\alpha 2$ domain. Within this region, CW3 and A24 differ only at position 173, which corresponds to lysine for CW3 and glutamic acid for A24. We found a clear correlation between recognition of P815 HLA transfectants and recognition of HLA peptides from this region. Some clones recognized either only CW3 or only A24 peptides, and others recognized both. For clones that recognized mutually exclusively CW3 or A24 peptides, we found that lysis could be inhibited by the presence of an excess of the homologous HLA peptide. Moreover, peptides from the homologous region of the endogenous class I antigens, K^d and D^d or L^d, could also compete with HLA peptides, whereas a peptide from the homologous region of HLA-B7 failed to compete. The apparent focus of the K^d-restricted CTL response of DBA/2 mice to P815 HLA transfectants to distinct epitopes located in a limited region of HLA and the ability of homologous HLA or H-2 peptides to compete for recognition are discussed in terms of possible models for the interaction of peptides, class I MHC molecules, and antigen-specific TCR.

Materials and Methods

Cells. The derivation and characterization of P815 cells transfected with HLA-CW3, HLA-A24, HLA-A3, or HLA-B7 genes were previously described (12, 16, 17). The isolation of CTL clones from DBA/2 mice immunized with P815 cells transfected with either HLA-CW3 or HLA-A24 genes is presented elsewhere (12, 14, 16). CTL clones are designated by the HLA gene expressed by the P815 transfectant used for immunization.

Peptide Synthesis and Purification. The modification of the Merrifield method (18) described in reference 14 was used. HPLC-purified peptides were >90% pure by analytical HPLC. Lyophilized peptides were dissolved in 0.7% sodium bicarbonate buffer or water and further diluted in DME containing 5% FCS.

Cytolytic Assay. P815 cells (10^6) were labeled with 150 μCi sodium [^{51}Cr]chromate as described (19) for 1 h at 37°C and washed three times. Labeled targets (2×10^3 in 50-μl volumes) were added to wells of V-bottomed microtiter plates containing 100-μl volumes of the appropriate peptide diluted in DME supplemented with 5% FCS and Hepes. CTL (6×10^3 cells) were added in 50-μl volumes. After a 4-h incubation at 37°C, the supernatants (100 μl) were harvested for counting. The percent specific lysis was calculated as: $100 \times$ [(experimental − spontaneous release)/(total − spontaneous release)]. For competition experiments, the cytolytic assay was modified as described in the figure legends.

Results

The K^d-restricted anti-HLA CTL clones used in this study were isolated from DBA/2 mice immunized with P815 cells transfected with either HLA-CW3 or HLA-A24 genes. Fig. 1 shows the specificity of four of these CTL for P815 targets transfected with different HLA genes. Clones CTL-CW3/10.1 (Fig. 1) and CTL-CW3/701.1 (16) lyse exclusively the CW3 transfectant, whereas the clone CTL-A24/10.1 lyses the A24 and A3 transfectants but not CW3 or B7.

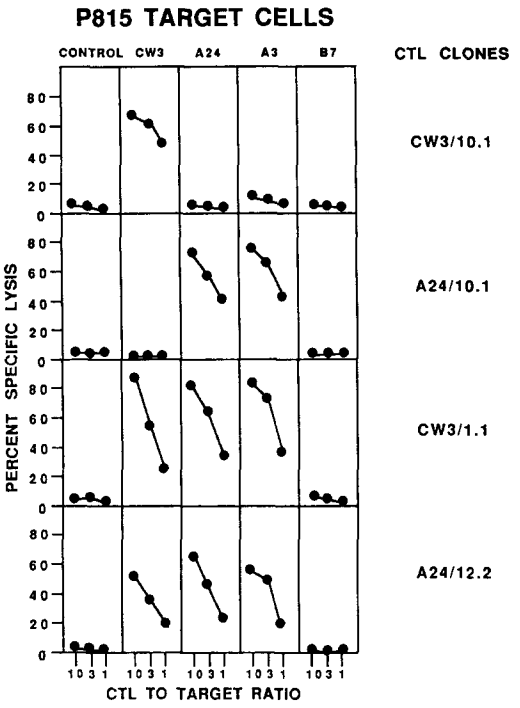

FIGURE 1. Lysis of P815-HLA transfectant target cells by K^d-restricted CTL clones. The cytolytic activity of CTL clones CW3/10.1, A24/10.1, CW3/1.1 and A24/12.2 was assessed against P815 HLA transfectant or control tk^+ target cells in a 4-h ^{51}Cr-release assay.

The other two clones CTL-CW3/1.1 and A24/12.2 are more broadly crossreactive in that they lyse CW3, A24, and A3 targets. None of the clones lyse HLA-B7 or control P815 targets.

We have previously shown that CTL clone CW3/10.1, but not clone A24/10.1, lyses P815 (HLA^-) target cells in the presence of synthetic peptides corresponding to HLA-CW3 amino acid residues 171–186 or 171–182 (14, 15). HLA-CW3 and HLA-A24 differ by only one amino acid residue within region 171–182 (Fig. 2). A peptide corresponding to this region of HLA-A24 was synthesized for comparison with the homologous CW3 peptide. In agreement with the pattern of recognition of P815-HLA transfectant target cells (Fig. 1), CTL clone CW3/10.1 recognized peptide CW3 171–182 but not the nearly identical homologous peptide A24 171–182 (Fig. 3). Conversely, CTL-A24/10.1 lysed

	170												182
CW3	Arg	Tyr	Leu	Lys	Asn	Gly	Lys	Glu	Thr	Leu	Gln	Arg	Ala
A24	–	–	–	Glu	–	–	–	–	–	–	–	–	–
A3	–	–	–	Glu	–	–	–	–	–	–	–	–	Thr
B7	–	–	–	Glu	–	–	–	Asp	Lys	–	Glu	–	–
K^d	–	–	–	Glu	Leu	–	Asn	–	–	–	Leu	–	Thr
D^d/L^d	–	–	–	Lys	–	–	Asn	Ala	–	–	Leu	–	Thr

FIGURE 2. Amino acid sequences of HLA-CW3, A24, A3, and B7 and of H-2-K^d, D^d, and L^d within region 170–182. The references for the sequences are given in reference 14.

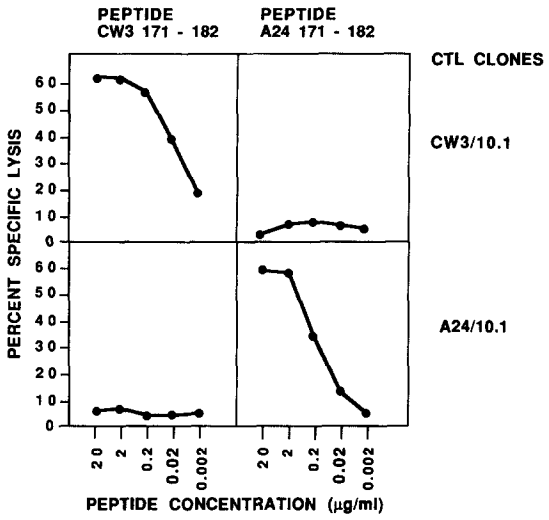

FIGURE 3. Mutually exclusive recognition of CW3 or A24 peptides (171–182) by CTL clones CW3/10.1 and A24/10.1. CTL were added at a 3:1 effector/target ratios to ^{51}Cr-labeled target cells in the presence of the indicated concentrations of HLA peptides. The assay was terminated after 4 h. The concentration of 20 μg/ml is equivalent to ~12 μM for both peptide CW3 171–182 and peptide A24 171–182.

P815 cells in the presence of the A24 but not the CW3 peptide (Fig. 3). Thus, these two CTL clones isolated from DBA/2 mice immunized with different P815-HLA transfectants apparently recognize epitopes from the same region of the HLA molecule.

To obtain more information on the fine specificity of the anti-HLA CTL clones, a series of homologous CW3 and A24 peptides varying in length from 10 to 15 amino acids and having a common COOH terminus at position 182 was synthesized (Fig. 4). For both CTL clone CW3/10.1 and clone CW3/701.1, peptide CW3 170–182 was the most efficient of the series in terms of the concentration required for lysis of P815 target cells (Fig. 5 *A* and *B*). Two longer peptides, CW3 169–182 and CW3 168–182, were also capable of inducing lysis but were less efficient than peptide CW3 170–182 by ~100-fold and 300-fold, respectively, for both clones. The two CTL clones clearly differed in recognition of a shorter peptide CW3 171–182. This peptide was ~300-fold and 10-fold less efficient than peptide CW3 170–182 for recognition by clone CW3/701.1

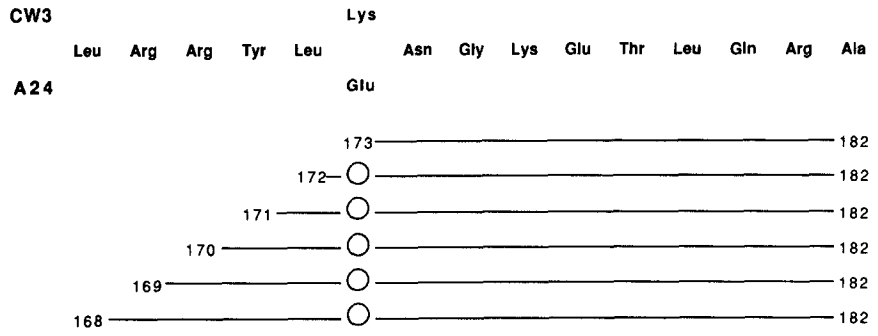

FIGURE 4. Amino acid sequences of a series of CW3 and A24 peptides synthesized for analysis in this study. Peptides corresponding to CW3 or A24 contained lysine or glutamic acid, respectively, at position 173.

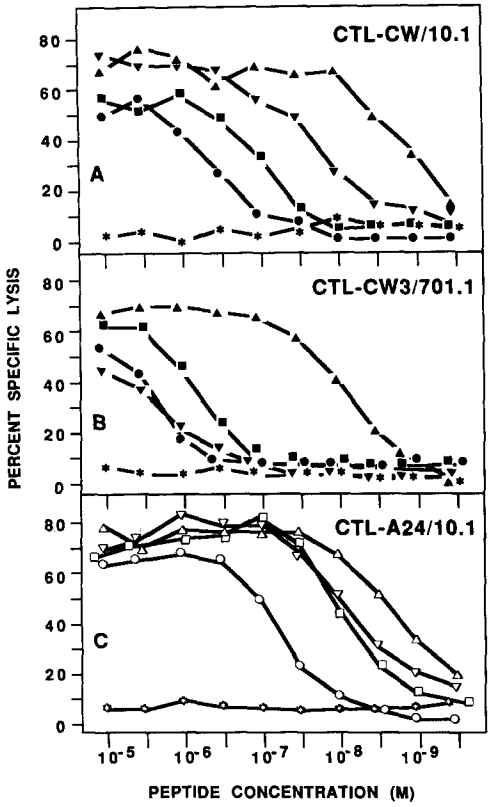

FIGURE 5. Recognition of CW3 or A24 peptides by CTL clones that lyse mutually exclusively P815 CW3 or A24 transfectant target cells. Cells from CTL clones CW3/10.1 (*A*), CW3/701.1 (*B*,) or A24/10.1 (*C*) were added to ^{51}Cr-labeled P815 target cells in the presence of the indicated concentrations of peptides CW3 172–182 (*), CW3 171–182 (▼), CW3 170–182 (▲), CW3 169–182 (■), CW3 168–182 (●), A24 172–182 (⊛), A24 171–182 (▽), A24 170–182 (△), A24 169–182 (□), and A24 168–182 (○). The effector/target ratio was 3:1 and the assay was terminated after 4 h.

and by clone CW3/10.1, respectively. Neither of the shortest CW3 peptides, corresponding to residues 172–182 and 173–182, nor any of the A24 peptides were recognized by either CTL clone (Fig. 5, *A* and *B* and data not shown). Within the A24 series, peptide A24 170–182 was optimal for CTL clone A24/10.1, compared with peptides A24 171–182 and A24 169–182 that were ~3-fold less efficient, and peptide A24 168–182 that was ~30-fold less efficient (Fig. 5 *C*). Neither of the shortest A24 peptides (172–182 and 173–182), nor any of the CW3 peptides was recognized by this clone (Fig. 5 *C* and data not shown).

In contrast to the CTL clones specific for either CW3 or A24, two different CTL clones that lyse both CW3 and A24 transfectant target cells recognized both CW3 and A24 peptide homologues (Fig. 6). However, it is noteworthy that each of these clones showed a clear preferential reactivity for the peptide within each CW3/A24 pair that corresponded to the immunizing HLA allele, as shown in Fig. 6 for the peptide pairs 170–182 and 168–182. Similar results were obtained for peptide pairs 171–182 and 169–182, whereas the shorter peptides 172–182 and 173–182 were not recognized (data not shown).

Thus, all of the clones analyzed in Figs. 5 and 6 appear to recognize closely related HLA epitopes that are located within the COOH-terminal end of the α2 domain. Substitution of residue 173 to correspond to CW3 or A24 affects recognition in two distinct ways, namely, either in a complete loss of recognition

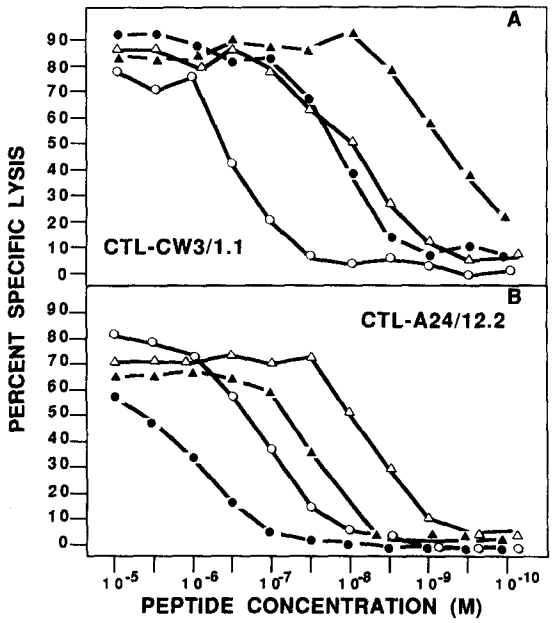

FIGURE 6. Recognition of CW3 and A24 peptides by CTL clones that lyse both P815-CW3 and P815-A24 transfectant target cells. Cells from CTL clones CW3/1.1 (*A*) or A24/12.1 (*B*) were added to ^{51}Cr-labeled P815 target cells in the presence of the indicated concentrations of peptides CW3 170–182 (▲), A24 170–182 (△), CW3 168–182 (●), and A24 168–182 (○). The effector/target ratio was 3:1 and the assay was terminated after 4 h.

as for clones CW3/10.1, CW3/701.1, and A24/10.1, or in a less efficient but nevertheless functional recognition as for clones CW3/1.1 and A24/12.2.

If the CW3 and A24 peptides bound to the same site(s), they might be expected to compete with each other for recognition. To test for competition, CTL clones that recognized exclusively CW3 or A24 peptides were assayed in the presence of the appropriate antigenic HLA peptide on P815 target cells preincubated with peptides from the homologous region of nonrecognized HLA alleles A24, CW3, or B7. Specific lysis of P815 target cells in the presence of peptide CW3 170–182 by CTL CW3/701.1 (Fig. 7) or by CTL-CW3/10.1 (data not shown) could clearly be inhibited by the homologous A24 peptide. Similarly, for clone A24/10.1, lysis in the presence of peptide A24 170–182 was inhibited by the CW3 homologue. In contrast, a peptide corresponding to the same region of HLA-B7 failed to compete with either the CW3 or the A24 peptide. None of the CTL clones used in this study lyses P815-B7 transfectant cells (Fig. 1 and reference 16) or P815 target cells incubated with peptide B7 170–182 (data not shown). Within region 170–182, HLA-B7 differs from A24 and CW3 by three and by four residues, respectively (Fig. 2).

We also tested two peptides corresponding to region 170–182 of K^d or D^d/L^d class I molecules that are endogenous to P815 cells. The K^d peptide 170–182 differs from the homologous CW3 and A24 peptides by five and four residues, respectively (Fig. 2). D^d and L^d molecules are identical to each other within region 170–182, but differ from K^d by three residues (Fig. 2). As shown in Fig. 8, peptides D^d/L^d 170–182 and K^d 170–182 were similarly efficient in competing with the homologous CW3 and A24 peptides. Lysis of P815 targets in the presence of either CW3 and A24 peptides from region 170–182 by cross-

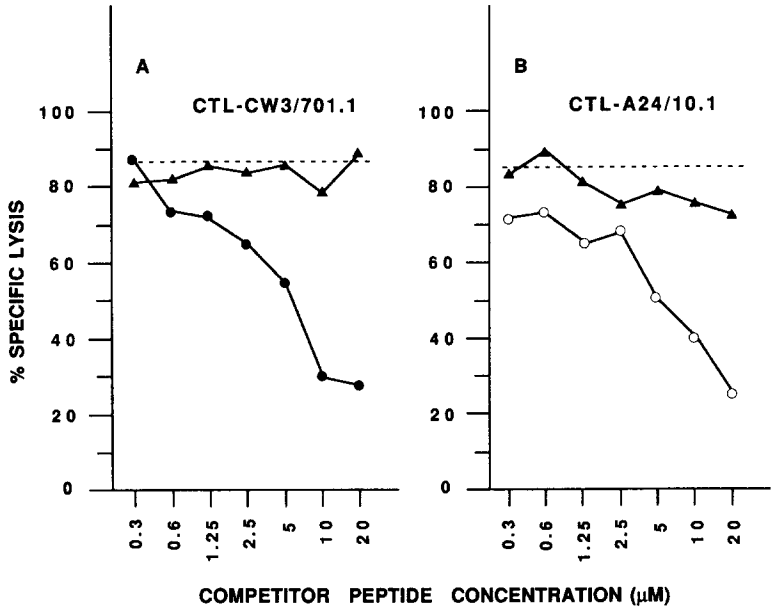

FIGURE 7. Inhibition of lysis by homologous HLA peptides. ^{51}Cr-labeled P815 target cells were incubated for 15 min at room temperature with various concentrations of competitor peptides A24 170–182 (●), CW3 170–182 (○), or B7 170–182 (▲) before addition of antigenic peptides CW3 170–182 (*A*) or A24 170–182 (*B*). After 15 min, cells from CTL clones CW3/701.1 (*A*) and A24/10.1 (*B*) were added at an effector/target ratio of 3:1. The assay was terminated after a 4-h incubation at 37°C. The final concentration of antigenic peptides was 0.03 μM for CW3 170–182 and 0.3 μM for A24 170–182, and that of competitor peptides varied from 0.3 to 20 μM as indicated in the figure. Lysis in the absence of competitor peptides is shown (– – –).

reactive clones CTL-CW3/1.1 and CTL-A24/12.2 could also be inhibited by peptides K^d 170–182 and D^d/L^d 170–182 (data not shown).

Several other peptides tested failed to inhibit lysis. For example, a synthetic peptide corresponding to residues 106–121 of HLA-CW3 had no effect on recognition of peptide CW3 170–182 by clones CW3/10.1 and CW3/701.1 or on recognition of peptide A24 170–182 by clone A24/10.1 (data not shown). Moreover, neither the horse cytochrome *c* (cyt *c*) peptide 39–53 known to be recognized by I-A^b-restricted T cells (20) nor the tetanus toxin peptide 1273–1284 recognized by DR-3-restricted human T cells (Demotz, S., A. Lanzavecchia, V. Eisel, H. Neimann, C. Widman, and G. Corradin, submitted for publication) could compete with peptides CW3 170–182 or A24 170–182 (Figs. 8 and 9 and data not shown). For two other peptides, horse cyt *c* 13–25 and the *Plasmodium falciparum* circumsporozoite peptide Tyr-(Asn-Ala-Asn-Pro)$_3$-Asn-Ala [Y(NANP)$_3$NA], recognized in the context of I-A^d (21) and I-A^b (22), respectively, the level of inhibition was minimal, compared with that obtained with peptides from region 170–182 of H-2-K^d or HLA-A24 (Fig. 9). Thus, it appears that peptides from region 170–182 of HLA alleles A24 and CW3 (but not B7) and of H-2 alleles K^d and D^d or L^d can compete with homologous HLA peptides for recognition by K^d-restricted CTL specific for CW3 or A24.

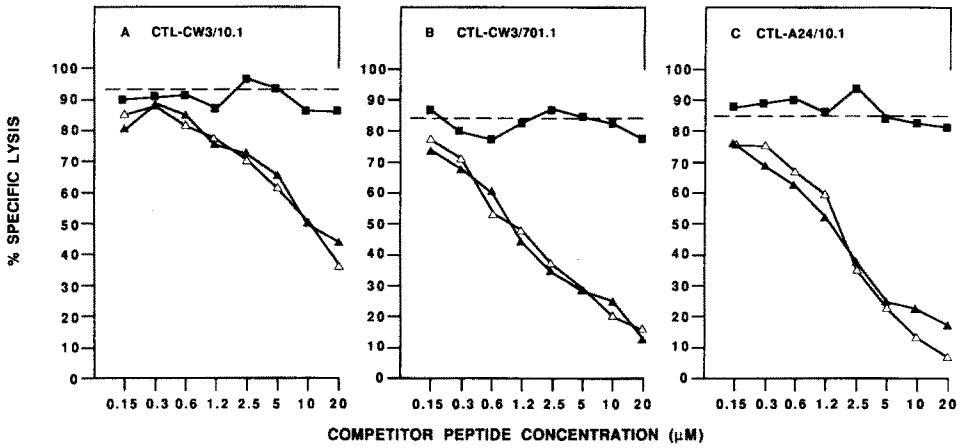

FIGURE 8. Comparison of K^d and D^d/L^d peptides 170–182 as competitors for homologous HLA peptides CW3 170–182 and A24 170–182. ^{51}Cr-labeled P815 target cells were incubated for 15 min at room temperature with various concentrations of competitor peptides K^d 170–182 (▲), D^d/L^d 170–182 (△), or tetanus toxin 1273–1284 (■) before addition of antigenic peptides CW3 170–182 (*A* and *B*) or A24 170–182 (C). After 15 min, cells from CTL clones CW3/10.1 (*A*), CW3/701.1 (*B*), and A24/10.1 (*C*) were added at an effector/target ratio of 3:1. The assay was terminated after a 4-h incubation at 37°C. The final concentration of HLA peptides was 0.1 μM and that of competitor peptides varied from 0.15 to 20 μM as indicated in the figure. Lysis in the absence of competitor is shown (— — —).

In preliminary experiments, we found that P815 targets preincubated with HLA peptides could be specifically lysed even if washed extensively before contact with CTL. It was therefore feasible to test separately the effect of a competing peptide on the target cells or on the CTL. P815 cells that were prein-

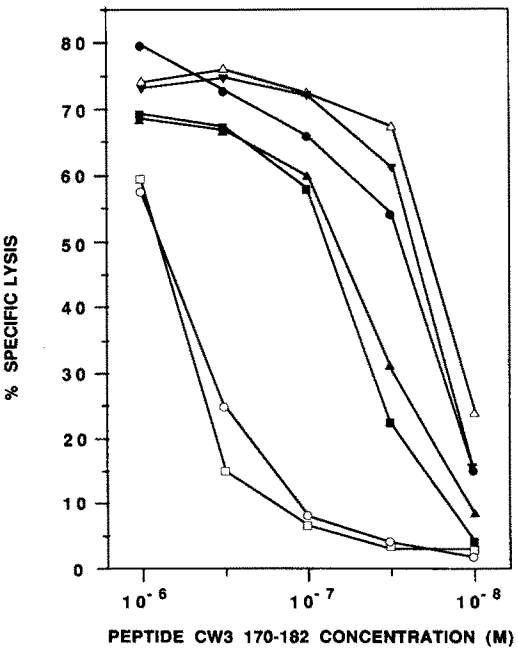

FIGURE 9. Inhibition by various peptides of the lysis of P815 cells in the presence of peptide CW3 170–182. ^{51}Cr-labeled P815 target cells were incubated for 15 min at room temperature with peptides A24 170–182 (○), K^d 170–182 (□), horse cyt *c* 39–53 (△) and 13–25 (▲), tetanus toxin 1273–1284 (▼), and $Y(NANP)_3NA$ (■) or with medium (●) as a control before addition of peptide CW3 170–182. After 15 min, cells from CTL clone CW3/701.1 were added at an effector/target ratio of 3:1. The final concentration of the competing peptides was 20 μM and that of peptide CW3 170–182 was as indicated in the figure. The assay was terminated after 4 h. The amino acid sequence of horse cyt *c* 13–25 is Lys-Cys-Ala-Gln-Cys-His-Thr-Val-Glu-Lys-Gly-Gly-Lys, that of horse cyt C 39–53 is Lys-Thr-Gly-Gln-Ala-Pro-Gly-Phe-Thr-Tyr-Thr-Asp-Ala-Gln-Lys, and that of tetanus toxoid 1273–1284 is Gly-Gln-Ile-Gly-Asn-Asp-Pro-Asn-Arg-Asp-Ile-Leu.

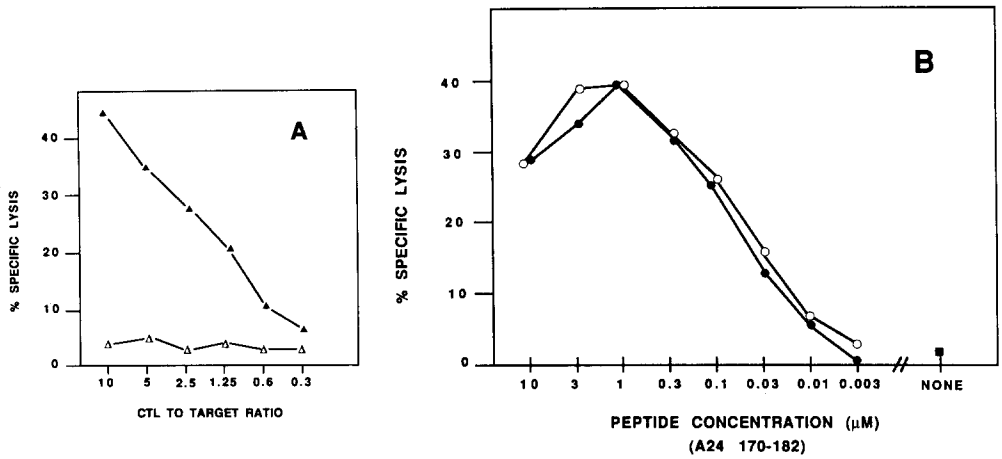

FIGURE 10. Peptide inhibition occurs at the level of the target cells not the CTL. (*A*) ^{51}Cr-labeled P815 cells (4 × 10^5 cells/ml) were incubated 1 h at 37°C with competitor peptide CW3 170–182 (200 μM) and antigenic peptide A24 170–182 (5 μM) (△) or with antigenic peptide A24 170–182 (5 μM) alone (▲) in DME containing 5% FCS. The cells were then washed three times, counted, and added (2,000 cells per well) to cells from CTL clone A24/10.1 at the CTL-to-target ratios indicated. The assay was terminated after 3 h. (*B*) Cells from CTL clone A24/10.1 (8 × 10^5 per ml) were incubated for 1 h at 37°C with competitor peptide CW3 170–182 (200 μM) (○) or with control medium (DME with 5% FCS) (●). The cells were washed three times, counted, and added to wells containing antigenic peptide A24 170–182 (at the final concentration indicated) and ^{51}Cr-labeled P815 cells (2,000 per well). The CTL-to-target ratio was 6:1. The assay was terminated after 3 h.

cubated with a mixture of antigenic (A24 170–182) and competing CW3 170–182) peptides and then washed were completely resistant to lysis by CTL-A24/10.1 compared with control target cells preincubated with the antigenic peptide alone (Fig. 10 *A*). In contrast, preincubation of the cells from the CTL clone with the competing peptide had no effect on their function, as measured by the concentration of antigenic peptide required for lysis (Fig. 10 *B*). Thus, in this system competition apparently occurs at the level of the target cells, not the CTL.

Discussion

We demonstrated previously (14–16) that antigens recognized by K^d-restricted CTL specific for P815-CW3 transfectants could be mimicked by synthetic HLA peptides. We show in the present study that K^d-restricted CTL clones from mice immunized with P815 cells transfected with a gene for another allele, HLA-A24, also recognize synthetic peptides. The P815 HLA transfectant clones used to immunize the DBA/2 mice from which these CTL clones were derived clearly express cell surface HLA molecules that can be detected by indirect immunofluorescence (17) or by RIA (12). In addition, HLA gene products expressed in P815 transfectants can function as target antigens for human alloreactive CTL (17) and as restriction elements for antiviral CTL (23, 24). It therefore seems remarkable that HLA is recognized as a nominal antigen in the context of H-2 class I antigens by CTL from syngeneic mice immunized with P815 HLA transfectants. However, for numerous viral systems, it has now been shown that CTL

can also be directed against antigens whose gene products are normally expressed internally. For example, antiinfluenza CTL can be directed against the viral NP (25) and antigens recognized by these CTL can be mimicked by synthetic peptides (11). Moreover, CTL specific for influenza hemagglutinin (HA) can lyse target cells transfected with a recombinant HA gene from which the leader sequence has been deleted and for which no cell surface expression can be detected (26). These observations suggest that for internally expressed gene products like NP, as well as for antigens like HLA and HA that are normally expressed at the cell surface, a fragmented or denatured form of the antigen may be presented by the appropriate MHC molecule for recognition by class I–restricted T cells.

From this report and our previous studies (14–16) it appears that the CTL response of DBA/2 mice to P815-CW3 or P815-A24 transfectants is predominantly K^d restricted and directed against epitopes located within the COOH-terminal end of the $\alpha 2$ domain. These epitopes can be defined by synthetic peptides from region 170–182. We demonstrated previously (14–16) that at least two CW3 epitopes could be distinguished in region 171–182 (or 171–186) by K^d-restricted CTL clones, based on recognition of the same CW3 peptides by CTL clone CW3/10.1 that lysed exclusively P815-CW3 transfectants and by clone CW3/1.1 that lysed both CW3 and A24 transfectant P815 target cells. In the present study, we have confirmed and extended these results. Within region 170–182, CW3 and A24 differ only at amino acid residue 173, which corresponds to lysine for CW3 and to glutamic acid for A24. Recognition of CW3 or A24 peptides corresponded exactly with recognition of P815-HLA transfectant target cells. For clones CW3/10.1, CW3/701.1, and A24/10.1, the CW3 and A24 peptides were recognized mutually exclusively, whereas for clones CW3/1.1 and A24/12.2 peptides corresponding to both alleles were recognized. In the latter case, however, the peptide corresponding to the immunizing allele was recognized more efficiently than its homologue. These results were obtained with a series of CW3 and A24 peptides having a common COOH terminus at position 182 and ranging from 12 (peptides 171–182) to 15 (peptides 168–182) amino acids in length. For all five CTL clones analyzed, the peptide corresponding to residues 170–182 of the immunizing HLA allele was the most efficient antigen for lysis. The demonstration of immunodominant epitopes within the same region of HLA alleles CW3 and A24 could be interpreted in support of the determinant selection hypothesis originally proposed for class II–restricted antigen recognition (3). According to this model, region 170–182 of these HLA molecules would contain features that favor interaction with the K^d molecule, and hence recognition by K^d-restricted cells.

The molecular details of antigen recognition by T cells have not yet been fully elucidated, even though genes encoding the α and β chains of the antigen-specific TCR heterodimer from a number of T cell clones and hybrids have been cloned and sequenced (27, 28). Moreover, the exact nature of antigens recognized by MHC-restricted T cells is not yet understood. For class II molecules, it has been demonsrated that antigenic peptides can directly and specifically interact with MHC molecules in vitro (29, 30) and that such complexes can efficiently stimulate IL-2 production by appropriate T cell hybridomas (31). For a number

of peptides for which direct binding to purified Ia molecules could be demonstrated, a correlation was found between the ability of peptides to compete with antigen for direct binding to class II molecules and their ability to inhibit antigen presentation in a stimulation assay (30, 32–34). In general, peptides restricted by the same haplotype were capable of competing with each other. These observations suggest that each class II molecule may contain a single binding site for peptide. In vitro binding studies have not yet been reported for class I MHC molecules; however, the demonstration that antigens recognized by class I–restricted CTL can be mimicked by synthetic peptides suggests that both classes of MHC molecules may function similarly by presenting antigenic fragments to T cells. The observations in the present study that both CW3 and A24 peptides can be recognized either mutually exclusively or in a crossreactive manner by K^d-restricted CTL clones suggests that both peptides are capable of interacting with the K^d molecule, and that for each peptide/K^d combination more than one T cell–defined epitope can be distinguished. Further interpretation of these results depends on whether the K^d molecule contains a unique site for peptide binding.

Differences in fine specificity between CTL clones that recognize the same HLA peptide might result from alternate "views" of the peptide MHC complex by different CTL clones (35), or from alternate conformations or orientations of the same peptide within the binding site. Alternatively, the HLA peptides may bind to more than one site, thus increasing the number of possible epitopes that would be formed. If both HLA peptides could interact with the same set of binding sites, they would still be expected to compete. Direct binding studies using purified K^d molecules and peptides, and X-ray crystallographic analysis of any complexes formed may eventually resolve these issues.

We present evidence in this report that peptides recognized by class I–restricted T cells can compete with each other in a functional way. We have evaluated competition in a cytolytic assay by determining either the concentration of antigenic peptide required to obtain lysis in the presence of a constant amount (20 μM) of competitor or the concentration of competing peptides required to reduce the level of lysis obtained by a limiting amount of antigenic peptide. Both approaches showed that peptide A24 170–182 could compete with peptide CW3 170–182 for recognition by CTL clones CW3/10.1 and CW3/701.1 and that, conversely, the CW3 peptide could compete with the A24 homologue for recognition by CTL clone A24/10.1. In the experiments presented in this report, the target cells were preincubated with the competitor peptide for 15 min before addition of the antigenic peptide. Similar results were obtained when the competitor and antigenic peptides were added simultaneously to the P815 target cells (data not shown). Peptides from the homologous region of the K^d or D^d/L^d molecules were also efficient competitors for both CW3 and A24 peptides. In contrast, a peptide from region 170–182 of the HLA-B7 MHC class I molecule could not compete efficiently with the CW3 or A24 peptide homologues. Of four unrelated peptides known to be recognized by class II–restricted T cells, two had no effect on recognition of HLA peptides, and two others [$Y(NANP)_3NA$ and horse cyt *c* 13–25] inhibited recognition only very inefficiently (Figs. 8 and 9). A simple interpretation of these results would

be that the homologous K^d, D^d/L^d, CW3, and A24 peptides (170–182) bind to the same site(s) on the K^d molecule, and that the B7 peptide fails to bind with sufficient affinity to compete. As might have been predicted, peptides corresponding to endogenous H-2 gene products were not themselves recognized as antigen by these HLA-specific CTL clones. It seems likely that at least one of the substituted residues in the H-2 peptides (compared with A24 or CW3) interferes with the formation of a recognizable epitope for these CTL clones. It should be possible to identify the critical residues by analysis of the appropriate substituted synthetic peptides.

HLA-B7 differs by amino acid residues 177, 178, and 180 from HLA-A24 within region 170–182 and by residues 173, 177, 178, and 180 from CW3 (Fig. 2). These results suggest that one or more residues at positions 177, 178, and 180 may be critical for interaction with the K^d molecule. We have recently found that a CW3 peptide corresponding to region 171–182 with a glutamic acid substitution (corresponding to the B7 residue) at position 180 was also recognized by CTL clones that recognized the unsubstituted CW3 peptide (unpublished observations). Moreover, the D^d/L^d peptide that contains an alanine residue position 177 (Fig. 2) can compete as efficiently as the K^d peptide in this system. Thus, it seems likely that the critical B7 substitution that results in lack of competition may be the lysine at position 178. P815 transfectants that express a cloned gene for HLA-B7 are not recognized as crossreactive targets by any of the CTL clones analyzed in this study (Fig. 1). Experiments are in progress to analyze the specificity and restriction pattern of the CTL response of DBA/2 mice to P815-B7 transfectants. From the failure of peptide B7 170–182 to compete with homologous CW3 and A24 peptides for recognition by K^d-restricted CTL, one might predict that the response against B7 would differ from that against the CW3 and A24 alleles.

Inhibition of lysis by HLA peptides has also been shown for human alloreactive CTL specific for HLA-A2 (36, 37). In the present study, we present evidence that inhibition of peptide-induced lysis by competing peptides occurs at the surface of the target cell (Fig. 10). With the demonstration that HLA peptides can be recognized by murine CTL in the context of mouse class I molecules (14), the possibility should be considered that alloreactive CTL might recognize MHC peptides in the context of the same or other MHC molecules. Other possibilities include recognition of non-MHC peptides or recognition of epitopes present on MHC molecules themselves. A clear understanding of antigen recognition by T cells may require in vitro analysis using purified MHC molecules, TCRs and peptides.

Summary

The specificity of peptide recognition by a number of K^d-restricted CTL clones specific for HLA-CW3 or HLA-A24 was investigated. The CTL clones were derived from DBA/2 ($H\text{-}2^d$) mice immunized with syngeneic P815 mouse cells transfected with genes encoding HLA-CW3 or HLA-A24 class I molecules. We had previously shown that CTL clones that lysed P815-CW3 transfectant target cells could lyse P815 (HLA^-) target cells incubated with synthetic CW3 peptides corresponding to the COOH-terminal end of the $\alpha 2$ domain. In the

present study, we found that K^d-restricted CTL clones that lysed P815-A24 transfectant target cells recognized a synthetic peptide from the same region (residues 170–182) of the A24 molecule. CW3 and A24 differ by only one amino acid within this region. Recognition of CW3 or A24 peptides corresponded exactly with lysis of P815-HLA transfectants both for clones that mutually exclusively lysed CW3 or A24 transfectant target cells and for CW3/A24 crossreactive CTL clones. The latter CTL clones that lysed both CW3 and A24 transfectant target cells showed a clear preference for the peptide corresponding to the immunizing HLA allele.

The homologous CW3 and A24 peptides could compete with each other for recognition, in contrast to a peptide from the same region of HLA-B7. Peptides from the corresponding region of the endogenous K^d and D^d/L^d molecules could also inhibit recognition of CW3 and A24 peptides. Competition with peptides apparently occurred at the level of the target cell. These results are consistent with a model whereby MHC class I molecules position protein fragments or peptides for specific recognition by T cells.

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