MHC Recognition by Hapten-Specific HLA-A2-Restricted CD8+ CTL

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T cell recognition by peptide-specific αβ TCRs involves not only recognition of the peptide, but also recognition of multiple molecular features on the surface of the MHC molecule to which the peptide has been bound. We have previously shown that TCRs that are specific for five different peptides presented by HLA-A2 recognize similar molecular features on the surface of the α1 and α2 helices of the HLA-A2 molecule. We next asked whether these same molecular features of the HLA-A2 molecule would be recognized by hapten-specific HLA-A2-restricted TCRs, given that hapten-specific T cells frequently show reduced MHC dependence/restriction. The results show that a panel of CD8+ CTL that are specific for the hapten DNP bound to two different peptides presented by HLA-A2 do the following: 1) show stringent MHC restriction, and 2) are largely affected by the same mutations on the HLA-A2 molecule that affected recognition by peptide-specific CTL. A small subset of this panel of CD8+ CTL can recognize a mutant HLA-A2 molecule in the absence of hapten. These data suggest that TCR recognition of a divergent repertoire of ligands presented by HLA-A2 is largely dependent upon common structural elements in the central portion of the peptide-binding site. The Journal of Immunology, 2003, 171: 2233–2241.

The specificity of T cell recognition of peptide/MHC complexes is determined by the V regions of αβ TCRs (1–3). Structural studies of αβ TCRs bound to complexes of peptide/MHC molecules have shown that peptide-specific TCRs make multiple contacts with the α1 and α2 helices of MHC class I molecules (4–10). Approximately two-thirds of the total surface area of class I MHC/peptide complexes contacted by TCRs is contributed by the MHC molecule (3). We have previously shown that the human A6 TCR recognizes human T cell leukemia virus type I (HTLV-I) Tax 11–19 peptide presented by HLA-A2 (11), and that this TCR binds to the HLA-A2 molecule via contacts on 6 aa on the α1 helix and 9 aa on the α2 helix (5). To assess the relative contribution of each of these 15 MHC contacts for A6 TCR recognition, alanine scanning mutagenesis of the HLA-A2 molecule at each of these contact residues was performed. The results showed that for A6 TCR recognition, only 3 aa (R65, K66, and A69) that are clustered on the α1 helix provided the critical interactions for the A6 TCR (12). When these same mutants at positions 65, 66, and 69 were assessed for their effects on recognition by a panel of 201 Tax/HLA-A2-specific CTL lines, each line was affected by at least 1 of these 3 aa substitutions (12). These results indicated that for all Tax/HLA-A2 TCRs examined, the area around amino acids R65, K66, and A69 provided a critical “focus” for MHC interaction.

Subsequent studies focused on determining whether TCRs specific for other peptides presented by HLA-A2 would also focus on this same area of the HLA-A2 molecule that was identified with the Tax-specific TCRs. CTL lines specific for four other peptides presented by HLA-A2 were analyzed for their capacity to recognize their specific peptides presented by the same panel of HLA-A2 mutants that was used for the Tax peptide-specific TCRs (13). The results demonstrated that most HLA-A2-restricted peptide-specific TCRs are functionally dependent upon similar molecular structures on the surface of the HLA-A2 molecule (13). These areas of focus were in the central portion of the peptide-binding site on the α1 helix at amino acid K66 and on the α2 helix at residue Q155. A similar TCR focus on particular areas of class I molecules also has been observed for murine peptide-specific and allospecific TCR recognition of H-2Kb and H-2Ld by other investigators (14, 15). Their studies demonstrated that mutations clustered around position 167 on the α2 helix and around position 82 on the α1 helix of Kd disrupted recognition by most Kd-restricted CTL (14). The common recognition pattern for Ld involved residues around positions 69, 72, and 76 on the α1 helix and positions 155 and 157 on the α2 helix (15). Taken together, these data suggest that peptide-specific TCRs that recognize Kb, Ld, and HLA-A2 have common recognition patterns for elements on the α1 and α2 helices of these class I molecules. Importantly, these recognition patterns are not identical.

In the present study, we asked whether this common recognition pattern for MHC exhibited by peptide-specific HLA-A2-restricted αβ TCRs would extend to other αβ TCRs that are specific for a different class of ligand, namely a hapten. Hapten-specific CD8+ CTL are thought to be the principle effector cells in chemical- and drug-induced contact hypersensitivity (16–18). CD8+ T cell responses to hapten such as trinitrophenyl (TNP) have been shown to be specific for hapten-modified peptides presented by MHC class I molecules (19–21). Unlike peptide-specific CTL, CD8+ hapten-specific CTL frequently show high levels of cross-reactivity on hapten-modified allogeneic targets (21–25). Franco et al. (21) induced TNP-specific CTL responses to TNP by coupling TNP to a lysine residue that was substituted within the sequences of known viral peptide epitopes presented by Kb. The results showed that many of these TNP-specific CTL were cross-reactive on TNP peptides presented by allo-MHC. Moreover, using a panel of Kb mutants with substitutions at potential TCR binding sites,
these investigators showed that unlike peptide-specific K<sup>b</sup>-restricted CTL, TNP-specific CD8<sup>+</sup> CTL recognition was relatively unaffected by any of these mutations (21). Collectively, these observations suggest that hapten-specific TCRs may exhibit a different pattern of MHC recognition than peptide-specific TCRs. To address this question for hapten-specific HLA-A2-restricted TCRs, we modified the approach of Franco et al. (21) and substituted a DNP-modified lysine residue at known TCR binding sites within two HLA-A2 peptide epitopes: influenza virus M1 58–66 and HTLV-I Tax 11–19. Both of these DNP-substituted peptides were able to induce DNP-peptide-specific CTL responses in vitro from the PBL of normal HLA-A2<sup>+</sup> donors. These DNP-peptide-specific CTL were then examined for HLA-A2 restriction and the effects on recognition by the same panel of HLA-A2 mutants that had been analyzed for peptide-specific CTL recognition (12, 13).

**Materials and Methods**

### Peptides

Two HLA-A2 binding peptides, influenza virus M1 58–66 (GILGFVFTL) (26) and HTLV-I Tax 11–19 (LLFGYPVYV) (27), and an HLA-A3-restricted influenza peptide nuclear protein 66–73 (ILRGSVAHK) (28) were synthesized with lysine at position 5 (Fig. 1; M1-5K, Tax-5K, A3NP-5K). These peptides were modified with DNP or benzyl (BENZ) coupled to the lysine residue (Fig. 1; M1-5K-DNP, M1-5K-BENZ and Tax-5K-DNP, Tax-5K-BENZ and A3NP-5K-DNP) by Princeton BioMolecules (Langhorne, PA). The HLA-A1-restricted influenza nuclear protein 44–52 peptide (CETLKLSDY) (28) with a naturally occurring lysine at position 5 was synthesized with (A1NP-DNP) and without (A1NP) DNP modification of that lysine (Fig. 1). Melanoma Ag recognized by T cells 1 (MART-1) 27–35 (AAGIGILTV) and human CMV matrix pp65 495–503 (NLVPMVATV) were synthesized and used as previously described (13). Peptides were HPLC purified and dissolved in PBS 50% DMSO at 1 mg/ml. Mass spectrum analysis of DNP-modified peptides confirmed their homogeneity.

**Generation and assay of DNP-peptide-specific CTL**

Leukapheresis of HLA-typed normal donors and human plasma were obtained from Department of Transfusion Medicine (National Institutes of Health, Bethesda, MD). PBL from nine normal HLA-A<sup>A</sup>0201 donors were cultured with M1-5K-DNP or Tax-5K-DNP (10 μg/ml) in upright 25-ml flasks (Nalge Nunc International, Rochester, NY) (12 × 10<sup>6</sup> PBL per flask) in 10 ml of culture medium (Iscove’s Modified Eagle’s Medium; Invitrogen, Grand Island, NY), 2 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (BioWhittaker, Walkersville, MD), 10% pooled human plasma. On day 3, 10 U/ml rIL-2 (Biological Resources Branch, National Cancer Institute, National Institutes of Health, Frederick, MD) was added to each flask. After 7 days of primary sensitization culture, responding cells were harvested and depleted of CD4<sup>+</sup> T cells using M-450 CD4 Dynabeads (Dynal Biotech, Lake Success, NY) as described by the manufacturer. CD4-depleted responder cells were then stimulated in secondary culture with autologous, irradiated (3000R) stimulator PBL pulsed with 10 μg/ml DNP peptides in upright 25-ml flasks (1 × 10<sup>6</sup> DNP responder cells and 9 × 10<sup>6</sup> stimulator cells/flask) in 10 ml of culture medium plus 10 U/ml rIL-2. Five days later, secondary responder cells were harvested and assayed for cytotoxicity (E:T ratio = 10:1) by a time-resolved fluorometric assay using HLA-A2-transfected Hmy2.C1R (29) target cells, as described previously (7). Responder cells from all donors exhibited stronger cytotoxicity on targets pulsed with the immunizing DNP peptide (10 nM) compared with unpulsed targets. These cells were restimulated in tertiary culture with autologous DNP-peptide-pulsed stimulator cells using the same regimen as was used with the secondary cultures. After 5 days, seven of the nine donors’ responder populations showed highly specific cytolytic activity on targets pulsed with the immunizing DNP peptide. These populations were placed into limiting dilution culture with DNP-peptide-pulsed allogeneic HLA-A2<sup>+</sup> PBL and 10 U/ml rIL-2 as previously described (11). CTL lines were selected based on lysis of targets pulsed with the immunizing DNP peptide and no lysis on targets pulsed with the corresponding unmodified peptide (M1-5K or Tax-5K). All selected CTL lines were analyzed for cell surface expression of CD4 and CD8 (Simultest; BD Biosciences, San Jose, CA) and αβ TCR (FITC-WT31, BD Biosciences) by flow cytometry with a FACSCalibur (BD Biosciences) as previously described (12). TCR Vβ expression was analyzed by PCR analysis as we have previously described in detail (11). CTL lines M1-DNP-1,3-7 and Tax-DNP-1,3 were generated from donor Q343; CTL lines M1-DNP-4,7 and Tax-DNP-4 were generated from donor Q338; CTL line Tax-DNP-5 was generated from donor Q337; and CTL lines M1-DNP-8,12 were generated from donor Q350.

A bulk culture line A1NP-DNP,1 was generated against the HLA-A1-binding A1NP-DNP peptide and bulk culture CTL line A3NP-DNP,1 was generated against the HLA-A3-binding A3NP-5-K-DNP peptide exactly as described above from the PBL of HLA-A1<sup>+</sup> donor Q343 and the PBL of HLA-A3<sup>+</sup> donor Q348, respectively.

**Generation and expression of HLA-A2 mutant transfectants**

Previously, a panel of HLA-A2 mutants (Table I) was created (12) and transfected into Hmy2.C1R cells (29). In this mutant panel, each of the HLA-A2 amino acids contacted by the Tax-specific A6 TCR was replaced with alanine (or glycine in cases of alanine in the wild type) by site-directed mutagenesis. All mutant transfectants showed cell surface expression of HLA-A2 at similar levels as wild-type HLA-A2, as detected by one or both HLA-A2-specific Abs (12). This mutant panel was used in the current study. HLA-A1- and HLA-A3-transfected Hmy2.C1R cells were generated and characterized as previously described (26, 30).

### Peptide binding to cell surface HLA-A2 molecules

A modification of the acid-strip procedure described by Storkus et al. (31) was used as previously described (32). HLA-A2 wild-type and mutant transfectants were incubated with 20 μg/ml brefeldin A (Sigma-Aldrich, St. Louis, MO) for 2 h at 37°C to inhibit the transport of newly synthesized cell-surface molecules to the cell surface. The cells were washed and resuspended in 0.13 M citric acid/PBS (pH 3.0), 0.5% human serum albumin, and 10 μg/ml human β<sub>2</sub>-microglobulin for 2 min on ice. The cells were washed with PBS, 0.5% human serum albumin, and 5 μg/ml human β<sub>2</sub>-microglobulin, and resuspended in the same buffer plus various concentrations of peptide and 2 μg/ml brefeldin A. The cells were incubated for 3 h at room temperature, and cell surface expression of HLA-A2 was quantified by indirect immunofluorescence with BB7.2 and analyzed by flow cytometry (13).

### Results

**Hapten specificity of DNP-peptide-induced CTL**

Hapten-specific CTL were induced from PBL of HLA-A<sup>A</sup>0201<sup>+</sup> donors. CTL lines were generated against two DNP-modified HLA-A2 binding peptides, influenza virus M1 58–66 peptide with...
a lysine substitution at position 5 (M1-5K-DNP) and HTLV-I Tax peptide 11–19 with a lysine substitution at position 5 (Tax-5K-DNP) (Fig. 1). The DNP-peptide-induced CTL lines were initially selected from limiting dilution cultures for their capacity to lyse HLA-A2-transfected Hmy2.C1R cells pulsed with the immunizing DNP peptide, but not with the unmodified peptide (M1-5K and Tax-5K, Fig. 1). CTL lines generated against M1-5K-DNP exhibited two different patterns of recognition when assayed on targets pulsed with these peptides (Fig. 2, A–D). CTL line M1-DNP.1 only lysed HLA-A2-transfected Hmy2.C1R cells pulsed with the immunizing M1-5K-DNP peptide, but neither the unmodified M1-5K peptide nor the Tax-5K-DNP peptide-pulsed cells were lysed (Fig. 2A). In contrast, CTL line M1-DNP.5 lysed these targets pulsed with both M1-5K-DNP and Tax-5K-DNP, but not with M1-5K (Fig. 2C) or Tax-5K (data not shown). The same two patterns of recognition were found with the CTL lines generated against Tax-5K-DNP, as is shown in Fig. 2, B and D. The results show that Tax-DNP.1 lysed only the HLA-A2-transfected

### Table 1.

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* More than 100-fold reduction in activity as quantified by the concentration of peptide required for half-maximal lysis.

* Cross-reactive on M1-5K-DNP and Tax-5K-DNP.

* None detectable.

**FIGURE 2.** Reactivity patterns of CTL lines generated against DNP peptides. A–D, CTL lines generated against either M1-5K-DNP or Tax-5K-DNP in limiting dilution culture were assayed against A2.1-Hmy2.C1R cells in the presence of 1000 nM indicated peptides. E:T ratio = 2.5:1. E and F, CTL lines generated against M1-5K-DNP (E) and Tax-5K-DNP (F) were assayed on A2.1-Hmy2.C1R targets pulsed with various concentrations of the indicated peptides. E:T ratio = 2.5:1.
Hmy2.C1R cells pulsed with the immunizing Tax-5K-DNP peptide, but not the unmodified Tax-5K peptide or the M1-5K-DNP peptide (Fig. 2B). The Tax-DNP.3 CTL line lysed targets pulsed with both Tax-5K-DNP and M1-5K-DNP, but not Tax-5K (Fig. 2D) or M1-5K (data not shown). In this initial panel, 12 DNP-peptide-specific CTL lines were isolated from three different HLA-A2 donors; seven lines lysed only those targets pulsed with the immunizing DNP-modified peptide, whereas five lines (three induced with M1-5K-DNP, two with Tax-5K-DNP) recognized targets pulsed with either one of the DNP-modified peptides. All CTL lines were CD8+ and CD4- and expressed αβ TCRs (WT31+) as analyzed by flow cytometry (data not shown). The TCR β-chains detectable in these CTL lines by PCR analysis are listed in Table I.

The TCRs expressed by the CTL lines that exhibited specificity only for the immunizing DNP peptide apparently recognized DNP
plus some component of the immunizing peptide, but not DNP alone. Fig. 2, E–F shows that among the remainder of the CTL lines that recognized both DNP-modified peptides, four of these five CTL lines showed comparable recognition of M1-5K-DNP and Tax-5K-DNP (representative results for two of these CTL lines are shown). In contrast, M1-DNP.7 showed a marked preference for the immunizing peptide (data not shown). To further examine the hapten specificity of these CTL lines, the CTL were assayed on HLA-A2-transfected Hmy2.C1R cells pulsed with the same lysine-substituted peptides coupled with a BENZ moiety (see Fig. 1). Representative results in Fig. 2, E–F shows that none of these cross-reactive CTL lines lysed targets pulsed with M1-5K-BENZ or Tax-5K-BENZ. Thus, these CTL lines displayed highly specific recognition of the DNP hapten bound by either M1-5K and/or Tax-5K.

**MHC recognition by DNP-peptide-induced CTL**

To determine whether these DNP-peptide-specific CTL recognized the hapten presented by HLA-A2 or were cross-reactive on allogeneic HLA molecules, the CTL lines were assayed on Hmy2.C1R cells that had been transfected with either HLA-A2 or HLA-A1 and pulsed with HLA-A2- and HLA-A1-binding (28) DNP peptides. The results for three representative CTL lines are shown in Fig. 3, A–C. No significant lysis was seen on the HLA-A1 targets pulsed with any of the DNP peptides, including lysis by one CTL line that cross-reacted on both M1-5K-DNP and Tax-5K-DNP (Fig. 3C). A CTL line generated against the A1NP-DNP peptide lysed HLA-A1-transfected cells pulsed with A1NP-DNP, but not A1NP, M1-5K-DNP, or Tax-5K-DNP (Fig. 3D), thus demonstrating clear DNP-peptide specificity and the presence of the DNP peptide on the surface of these HLA-A1+ target cells. These A1NP-DNP/HLA-A1-specific CTL also did not lyse HLA-A2-transfected targets pulsed with any of the DNP peptides (Fig. 3D).

To further test the stringency of MHC restriction of these DNP-peptide-specific CTL lines, we assayed them on HLA-A3-transfected targets pulsed with the HLA-A3-binding (28) peptide A3NP-5K-DNP as well as Tax-5K-DNP and M1-5K-DNP. Representative results for three CTL lines are shown in Fig. 3, E–G. All CTL lines showed strong lysis of the HLA-A2-transfected targets pulsed with the immunizing DNP peptide, whereas none of the CTL lines showed >7% lysis of the HLA-A3-transfected targets pulsed with any of the DNP peptides. A CTL line generated against the A3NP-5K-DNP peptide lysed HLA-A3- but not HLA-A2-transfected cells pulsed with A3NP-5K-DNP, but not A3NP-5K, M1-5K-DNP, or Tax-5K-DNP (Fig. 3H), thus demonstrating DNP-peptide specificity and HLA-A3 restriction. Collectively these data show that all of the DNP-peptide-specific CTL lines exhibited little or no cross-reactivity on other HLA-A alleles.

**Recognition of HLA-A2 mutants by DNP-peptide/HLA-A2-specific CTL**

The crystallographic structure of the trimolecular complex of the Tax peptide-specific A6 TCR complexed with Tax peptide/HLA-A2 revealed that the TCR contacted 15 aa on the surface of the HLA-A2 molecule, 6 on the α1 helix and 9 on the α2 helix (5). In those studies, alanine scanning mutagenesis was performed at each of those positions, and the results suggested that common elements (K66 and Q155) on the surface of the HLA-A2 molecule were present that were recognized by the majority of HLA-A2-restricted peptide-specific TCRs (12, 13). In the present study, we

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**FIGURE 4.** Effects of mutations in the α1- and α2-helix of HLA-A2 on recognition by DNP-peptide-specific CTL. A and B, Two representative DNP-peptide-specific CTL lines were assayed on wild-type A2.1 and the indicated six different α1-helix mutant transfectants in the presence of increasing concentrations of the immunizing DNP peptide. C and D, Two representative DNP-peptide-specific CTL lines were assayed on wild-type A2.1 and the indicated nine different α2-helix mutant transfectants in the presence of increasing concentrations of the immunizing DNP peptide. E:T ratio = 2.5:1.
wished to know whether a similar pattern of HLA-A2 recognition would be found for DNP-peptide-specific HLA-A2-restricted TCRs. Therefore, we assayed this panel of CTL on the same set of HLA-A2 mutant transfectants pulsed with the immunizing DNP peptide. Representative results for CTL recognition of the α1 helix mutants are shown in Fig. 4, A and B; and results for the α2 helix mutants are shown in Fig. 4, C and D. All results for the most negative effects (>100-fold reduction in activity as quantified by the amount of peptide required for half-maximal lysis) are summarized in Table I. All of the DNP-peptide-specific CTL lines were negatively affected by at least one HLA-A2 mutant, but most CTL lines were affected by only a few of the mutants (Table I). For the α1 helix mutations (Fig. 4, A and B), 9 of 12 of these CTL lines were negatively affected by the K66A mutation (e.g., M1-DNP.3). Six of the 12 CTL lines were also negatively affected by A69G, whereas the rest were unaffected by A69G (Table I). Mutations R65A, K68A, and A149G also affected DNP-peptide recognition by a subset of the CTL lines (Table I).

For the α2 helix mutations (Fig. 4, C and D), 8 of 12 showed a strong negative effect by Q155A (e.g., M1-DNP.3), whereas the others showed little or no effect of the Q155A mutation. Moreover, mutations at H151A, A158G, T163A, and E166A also produced strong negative effects by selected DNP-peptide-specific CTL lines (Fig. 4C and Table I).

These results demonstrate that the majority of our panel of DNP-peptide-specific CTL lines are affected by the same mutations (K66A and Q155A) that affected most of the five different peptide-specific CTL lines previously examined. Mutations at these positions affected some peptide-specific CTL, with one exception: the effect of H151A on M1-DNP.6 was never observed for any of the peptide-specific CTL lines. A comparison of the effects of this panel of HLA-A2 mutants on DNP-peptide-specific CTL lines that were either cross-reactive on the other DNP peptide or not cross-reactive (Table I) did not reveal any obvious differences in the patterns of mutational effects, nor was there a correlation with TCR Vβ expression.

DNP peptide binding to cell surface A2.1, K66A, and Q155A
To determine whether the predominant negative effects of K66A and Q155A for presentation of DNP peptides could be caused by negative effects on peptide binding, we removed endogenous peptides from these cell surface molecules by acid stripping and then incubated the cells with no peptide or Tax-5K-DNP and M1-5K-DNP peptides. Peptide binding was quantified by the measurement of mean fluorescence intensity (MFI) following binding of the HLA-A2-specific BB7.2 Ab. The results shown in Fig. 5A demonstrate that cell surface HLA-A2.1, K66A, and Q155A molecules are reconstituted to roughly the same extent by these peptides, indicating that these molecules present comparable levels of peptide/MHC complexes.

To determine whether cell surface complexes with DNP peptides were less stable on K66A and Q155A compared with wild-type, cell surface molecules were acid stripped and reconstituted with 10 μM Tax-5K-DNP or M1-5K-DNP. Following reconstitution, the cells were washed and incubated at 37°C for various times in the absence of exogenous peptides to permit the Tax/A2 complexes to dissociate. Quantitation of cell surface complexes was measured by reactivity with BB7.2. The results (Fig. 5B) show that very little Tax-5K-DNP complexes disappear up to 3 h after removal of exogenous peptide from either K66A, Q155A, or wild-type HLA-A2. Approximately half of the M1-5K-DNP complexes were absent from HLA-A2 by 3 h, but neither K66A nor Q155A were appreciably lower than HLA-A2. These results indicate that there were little if any detectable differences in DNP-peptide binding by these mutants compared with wild type.

Recognition of HLA-A2 mutants in the absence of exogenous cognate peptide
During the course of screening DNP-peptide-specific CTL lines on this panel of mutant transfectants, two M1-5K-DNP-specific CTL lines (M1-DNP.11 and M1-DNP.12) were observed to lyse Q155A in the absence of added DNP peptide. No other mutant or wild-type transfectants were lysed in the absence of exogenous peptide by these two CTL lines (representative results are shown in Fig. 6). One possible explanation for these results is that these CTL lines cross-react on an endogenous peptide(s) that is differentially presented by Q155A, and none of the other mutants or wild-type HLA-A2 shared this property with Q155A. To examine this issue and the potential role of endogenous peptide requirements for recognition of Q155A, Q155A cell surface molecules were acid stripped of endogenous peptides and reconstituted with four different HLA-A2-binding peptides. Flow cytometric analysis of cells reconstituted with no peptide revealed a 1–2 log reduction in cell surface expression of Q155A (histograms are shown in Fig. 7A; MFI values for each curve are plotted in Fig. 7B). Reconstitution with individual HLA-A2 binding peptides resulted in partial reconstitution of cell surface expression. Despite the marked reduction in cell surface expression of Q155A following acid stripping, these cells showed no reduction in lysis by M1-DNP.12, and no effect on lysis was observed upon the addition of the HLA-A2-binding peptides (Fig. 7C). These results suggest that DNP-peptide-specific TCR recognition of Q155A is either peptide independent, or it involves peptide(s) that are not removed by the acid-stripping procedure.

**FIGURE 5.** Peptide binding by cell surface HLA-A2.1, K66A, and Q155A. A, Levels of cell surface expression following acid stripping and reconstitution with 10 μM and 1 μM Tax-5K-DNP and M1-5K-DNP peptides. The y-axis shows MFI values following incubation with BB7.2. B, Levels of cell surface expression following acid stripping, reconstitution with 10 μM Tax-5K-DNP and M1-5K-DNP peptides, washing, and incubation in the absence of exogenous peptide at 37°C for the indicated periods of time. The y-axis shows MFI values following staining with BB7.2.
Discussion

In this study we sought to examine the patterns of MHC recognition by hapten-specific CTL. In addition, we wished to compare these recognition patterns with those exhibited by peptide-specific CTL. Our results demonstrate that human CD8+ αβ TCR+ DNP-peptide/HLA-A2-specific CTL exhibit stringent MHC restriction, and they are largely affected by the same mutations on the surface of the HLA-A2 molecule that affected recognition by peptide-specific CTL. (Such stringent MHC restriction was also exhibited by HLA-A1- and HLA-A3-restricted DNP-peptide-specific CTL.) These data suggest that TCR recognition of a divergent repertoire of ligands presented by HLA-A2 is largely dependent upon common structural elements in the central portion of the peptide binding site that are recognized by most HLA-A2-restricted TCRs.

These findings with human DNP-peptide-specific T cell recognition differ markedly from the findings of Franco et al. (21) for murine TNP-peptide/Kb-specific T cells. Their results showed that anti-TNP-peptide-specific CTL could recognize TNP peptides presented by many of the Kb molecules with mutations at potential TCR contact sites that failed to present peptide to many peptide-specific CTL clones. This capacity of the anti-TNP-peptide clones to be less dependent on the integrity of the potential TCR contact residues was apparently attributed to the fact that the anti-TNP-peptide clones had TCRs that were ~100-fold higher avidity than the peptide-specific clones, based on the amount of peptide required to sensitize target cells for lysis. In contrast, our human anti-DNP-peptide-specific CTL did not exhibit significantly higher avidity than our peptide-specific CTL based on the same criteria of avidity (compare the peptide titration curves in Figs. 2 and 4 with those in Figs. 1–3 of Ref. 13). Thus, the stringent MHC restriction and susceptibility to mutations at common TCR contact sites observed with our DNP-peptide-specific CTL may be attributed to


FIGURE 7. Acid stripping of cell surface Q155A, reconstitution with HLA-A2-binding peptides, and effects on recognition by M1-DNP.12. A, Q155A transfectants were acid stripped and reconstituted with 5 μM peptides. Cell surface expression was determined by staining with anti-HLA-A2 Ab BB7.2. FACS histograms are white, no treatment; green, acid stripped; then acid stripped and reconstituted with no peptide (pink), melanoma Ag recognized by T cells-1 (brown), pp65 (orange), and M1-5K-DNP (blue). B, Cell surface expression is expressed as MFI values on the y-axis. C, Lysis of the same Q155A cell populations in A and B by CTL line M1-DNP.12. E:T ratio = 5:1. No Rx, cells before acid stripping.
the fact that these T cells are of lower overall avidity than the murine TNP-peptide-specific T cells. One possible reason for this difference is that the DNP-peptide-specific T cells were generated from PBL obtained from donors who were not, to our knowledge, previously primed in vivo to DNP peptides, whereas the mice were primed in vivo with TNP peptides in adjuvant. Alternatively, there could be differences between DNP-peptide- and TNP-peptide-specific TCRs; the additional nitro group of TNP may select for higher affinity TCRs. The DNP-peptide-specific CTL clearly recognize these nitro groups, because they did not cross-react on the BENZ peptide that lacks these groups.

Stockl et al. (33) previously described MHC-independent recognition by human TNP-specific, HLA-A1-restricted CD8+ αβ TCR+ CTL. These TNP-specific CTL were generated against directly TNP-modified autologous cells and were shown to recognize MHC-incompatible or MHC class I null targets only when modified with high levels of TNP (5 mM). One of the target molecules on these target cells was identified as CD39, a cell surface ectonucleotidase that contains 26 lysine residues in its extracellular domain and is present at the cell surface as a homotetramer (34). Thus, CD39 could present many potential TNP modification sites that would present a very high concentration of hapten. Hapten-specific TCR interaction with such a structure may overcome the requirement for MHC interaction. We have observed that a subset of our DNP-peptide-specific CTL can recognize HLA-A2-negative targets modified with 5 mM dinitrobenzene sulfonic acid, but we have not been able to block this recognition with anti-CD39 Ab (S. Gagnon, unpublished data). Thus, in our study the potential target of these DNP-peptide-specific TCRs on DNP-modified HLA-A2-negative targets awaits identification, but the ability of these DNP-peptide-specific CTL to lyse DNP-modified HLA-A2 negative targets also indicates that hapten/MHC-specific αβ TCRs can recognize hapten in the absence of the cognate MHC. This form of MHC-independent recognition of hapten-modified cells could account for the high degree of MHC-unrestricted recognition previously observed on hapten-modified target cells by others (21–25).

A small subset of our DNP-peptide-specific CTL lines were able to recognize Q155A in the absence of exogenous peptide, but none of the other mutants or wild-type targets. This pattern of recognition is exactly the same as that pattern observed for 10 of 12 pp65 peptide/A2-specific CTL lines tested (13). Similar to the DNP-peptide-specific CTL, acid-strip removal of endogenous peptides from Q155A did not inhibit recognition by the pp65 CTL lines, and addition of HLA-A2-binding peptides also did not inhibit recognition. The failure of noncognate peptide or acid stripping to eliminate recognition in both cases could mean that the endogenous peptide was very tightly bound and is recognized with high affinity. Alternatively, these results mean that recognition is specific for a particular conformation of HLA-A2 that is imparted by the Q155A mutation and is largely peptide independent. Perhaps the substitution of alanine for glutamine at position 155 replaces an unfavorable interaction with a favorable or neutral one that permits TCR binding to proceed with a high enough affinity that lowering the levels of cell surface expression of Q155A by 10-fold did not reduce the level of lysis (Fig. 7). Despite the uncertainty of the specificity of recognition of Q155A by DNP-peptide-specific and pp65-peptide-specific CTL, these findings further illustrate the strikingly similar MHC components that can be recognized by both hapten- and peptide-specific TCRs. This type of cross-reactive recognition by TCRs may be analogous to other specific binding molecules that can bind multiple ligands with composition, sizes, and shapes that are different from those of the natural-binding protein ligands (35).

References


