AN IMMUNODOMINANT EPITOPE PRESENT IN MULTIPLE CLASS I MHC MOLECULES AND RECOGNIZED BY CYTOTOXIC T LYMPHOCYTES

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The antigen specificity of antiviral CTL is thought to be the result of TCR recognition of an antigenic polypeptide seen in association with a self class I MHC molecule (1–3). The CTL epitope is controlled by the antigen, since CTL responses to cell surface viral proteins discriminate between related viral antigens (4, 5) and the class I molecule itself (6).

In addition to antigen-specific MHC-restricted CTL, CTL can be readily generated against allogeneic class I molecules. In general, ~90% of CTL directed against a given alloantigen are specific for that molecule; whereas <10% of CTL clones derived from a bulk population crossreact on other class I molecules (7, 8). This finding is not unexpected since class I alloantigens differ from each other in ~10% of their amino acids. However, the same exquisite specificity is noted when CTL generated against the H-2Kb molecule are tested against H-2Kb mutant antigens that differ from the parental type by only one to three amino acids (9–12). For example, the H-2Kbm1 mutant differs from the wild-type H-2Kb molecule at amino acid positions 152, 155, and 156 (13, 14). As a result of this change <25% of anti-H-2Kb CTL clones recognize this molecule (15). Similar findings are noted with CTL directed against subtypes of HLA molecules (16, 17).

While most alloreactive CTL demonstrate this high degree of specificity, some show greater than expected crossreactivity on third-party targets. Thus, anti-H-2d CTL readily crossreact with H-2a antigens (18). Although the B6 anti-H-2Kbm1 response discriminates between other H-2Kb mutant molecules, it has been demonstrated that these effector cells crossreact with other H-2 molecules (19). The nature of the antigenic determinants recognized by alloreactive T cells and the mechanism by which this fine specificity is achieved are poorly understood.

Recently, the structure of the class I antigen HLA-A2 was shown to comprise two α-helical regions (20, 21). One of these regions is contained in the α1 domain (amino acids 50–84), the other in the α2 domain (amino acids 138–173). Both traverse a

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region of eight antiparallel β-pleated sheets. Together, these α-helical regions form a cleft on the apical surface of the molecule that is believed to be the site for binding peptides. This raises the possibility that alloreactive CTL recognize amino acids from a peptide together with the foreign class I α helices.

Whether determinants recognized by alloreactive CTL can be identified independently in the α1 and α2 domains, or if together they form a conformational or non-linear epitope, has been addressed by creating domain-shuffled class I molecules in which the α1 and α2 domains are derived from two different class I molecules (22-28). In most cases these domain-shuffled molecules are poorly recognized by CTL that have been sensitized against either native molecule. Thus, these data would suggest that alloreactive CTL recognize nonlinear epitopes. We have recently reported (29) the generation of bulk-cultured CTL directed against determinants encoded by the α1 and α2 extracellular domains of Q10d. These CTL crossreact extensively on three different class I molecules from the H-2d haplotype (30). In this report we provide evidence that this Q10-encoded epitope is controlled in part by three amino acids in the α2-encoded helix. However, recognition of this epitope is dependent on specific amino acids in the α1 domain.

Materials and Methods

Mice. C57BL/6, H-2Kbm1, C57BL/10, B10.D2(R103), B10.A(3R), (C3H × B6.K)F1, (C3H × H-2Kbm1)F1, (C3H × BALB/c)F1, and (C3H × BALB/c-H-2kbm1)F1 mice were bred and maintained in our colony at the University of Texas Southwestern Medical Center at Dallas.

Maintenance of Transfected Cell Lines. Identification of the Q10d gene, construction of the Q10d/Ld exon-shuffled class I gene, and transfection into L cells has been described previously (31). The Q10d/Ld- and HSV-tk-expressing L cell lines were obtained from Drs. I. Stroynowski and L. Hood at the California Institute of Technology, Pasadena, CA. Oligonucleotide-directed mutagenesis of the H-2K6 gene to produce the revertant genes KITry (designated Glu(152) in this report) and KAla (designated Arg-Leu(155-156) in this report) and transfection of these genes into L cells have been described previously (32). Generation of the mutant H-2Ld genes M66, M70, and M73 and transfection into L cells have been described (33). The H-2Ld-, M66-, M70-, M73-, and H-2Dd-expressing L cell lines were obtained from Drs. D. Koeller and K. Ozato at the National Institutes of Health, Bethesda, MD. Transfected L cells were maintained in α-MEM (Irvine Scientific, Santa Ana, CA) containing 5% FCS, glutamine, and gentamicin. L cells were selected and maintained in medium containing HAT.

Generation and Assay of CTL Activity. Mice were primed in vivo with 10<sup>7</sup> Q10d/Ld-expressing L cells or 3 × 10<sup>7</sup> spleen cells intraperitoneally. After an interval of 3 wk to 3 mo, 5 × 10<sup>6</sup> spleen cells from primed mice were stimulated in vitro with 10<sup>6</sup> irradiated (10,000 rad) Q10d/Ld L cells or 5 × 10<sup>6</sup> irradiated spleen cells (2,000 rad). After 5 d of culture, effector cell activity was tested using <sup>51</sup>Cr-labeled L cells in a standard 4-h chromium-release assay. Specific release equals the percentage of release of isotope from target cells in the presence of effector cells minus spontaneous release. Spontaneous release from L cell targets ranged between 5 and 15%, and from Con A lymphoblasts targets between 10 and 20%. SEM of triplicate samples did not exceed 2%.

Cloning of CTL. Anti-Q10 CTL clones were generated by priming (C3H × B6.K)F1 mice intraperitoneally with 10<sup>7</sup> Q10d/Ld L cells, followed by restimulation with the same L cells in vitro. After 7 d of culture the cells were placed into limiting dilution at multiple cell/well concentrations in 96-well plates with 10<sup>5</sup> irradiated H-2Kbm1 or BALB/c spleen cells. Cultures contained a final concentration of 25% rat Con A supernatant with α-methylmannoside. Clones were picked, expanded, and subcloned at limiting dilution.
Results

Anti-Q10 CTL Recognize H-2K\textsuperscript{bml}. H-2K\textsuperscript{bml} is a mutant gene derived from H-2K\textsuperscript{b}. The mutation involves changes in 7 bp that encode amino acid residues 152, 155, and 156. It has been suggested that this mutant may be the result of a gene conversion event between Q10\textsuperscript{d} and H-2K\textsuperscript{b} since the former gene shares H-2K\textsuperscript{bml} nucleotides in the region where the mutation occurred (14, 34–36). Q10 is a secreted class I molecule and cannot be tested directly as a target for CTL (36–38). However, by shuffling the first three exons of Q10\textsuperscript{d} with the latter five exons of H-2L\textsuperscript{d}, a hybrid molecule can be expressed on the cell membrane of transfected L cells containing the α1 and α2 domains of Q10 together with the carboxy-end of the molecule derived from H-2L\textsuperscript{d} (31). We recently showed CTL activity can be generated against this molecule and it is specific for determinants controlled by the α1 and α2 and not the H-2L\textsuperscript{d}-encoded α3 domain (29, 30). Since Q10 shares the H-2K\textsuperscript{bml} mutant amino acids at positions 152, 155, and 156 (see Table III), we tested whether anti-Q10 CTL would crossreact on H-2K\textsuperscript{bml}.

Anti-Q10 CTL were generated by stimulating spleen cells from (C3H × B6.K1)F\textsubscript{1} (K\textsuperscript{D^a/D^b}) mice with L cells (H-2\textsuperscript{k}) expressing the Q10\textsuperscript{d}/L\textsuperscript{d} hybrid molecule. The resulting CTL were tested for reactivity against Q10\textsuperscript{d}/L\textsuperscript{d}-transfected L cells, H-2K\textsuperscript{bml} lymphoblasts, or target cells derived from a panel of H-2K\textsuperscript{b} mutant strains including H-2K\textsuperscript{bml,5,6,8,10,11}. These other mutant strains have amino acid substitutions in the H-2K\textsuperscript{b} molecule at sites that differ from H-2K\textsuperscript{bml} (9, 10). The data in Fig. 1 (left panel) show that anti-Q10 CTL mediate specific lysis against Q10-transfected L cells with only weak lysis of L cells transfected with the tk gene, as previously described (29, 30). The same effector cells also display strong lytic activity against H-2K\textsuperscript{bml} targets, while little or no reactivity is detected on any of the other H-2K\textsuperscript{b} mutants (Fig. 1, right panel). Thus, anti-Q10-specific CTL crossreact on the H-2K\textsuperscript{bml} molecule that shares an alanine and two tyrosines at amino acid positions 152, 155, and 156, respectively, with Q10 (see Table III).

![Figure 1](image-url)
B6 Anti-H-2K\textsuperscript{bm1} CTL Crossreact with Q10\textsuperscript{d}/L\textsuperscript{d} and H-2L\textsuperscript{d}. The above data demonstrate antigenic crossreactivity between Q10\textsuperscript{d} and H-2K\textsuperscript{bm1}. In addition, crossreactivity between H-2K\textsuperscript{bm1} and a D end-encoded molecule from the H-2\textsuperscript{d} haplotype has been observed (39). It has also been noted that the H-2L\textsuperscript{d} molecule has the same sequence as H-2K\textsuperscript{bm1} at the mutant amino acid positions 152, 155, and 156 and evidence has been provided that anti-H-2L\textsuperscript{d} CTL crossreact on H-2K\textsuperscript{bm1} (40, 41). We previously showed that anti-H-2L\textsuperscript{d} CTL crossreact on Q10\textsuperscript{d} (29). Thus, anti-H-2L\textsuperscript{d} CTL appear to have a subset of cells capable of recognizing both H-2K\textsuperscript{bm1} and Q10\textsuperscript{d}. The reactivity of B6 anti-H-2K\textsuperscript{bm1} CTL on H-2L\textsuperscript{d} targets has been examined using a monolayer absorption technique and two subpopulations of CTL were detected directed at H-2\textsuperscript{d} targets (19). One apparently recognized H-2L\textsuperscript{d} while the second recognized another H-2\textsuperscript{d}-encoded molecule, possibly H-2K\textsuperscript{d}.

Based on the above studies, we reasoned that B6 anti-H-2K\textsuperscript{bm1} CTL would crossreact on Q10\textsuperscript{d} and thus tested these effector cells for reactivity on H-2K\textsuperscript{b}, H-2K\textsuperscript{bm1}, H-2L\textsuperscript{d}, and Q10\textsuperscript{d}/L\textsuperscript{d} L cells. B6 anti-H-2K\textsuperscript{bm1} CTL specifically lyse H-2K\textsuperscript{bm1} L cells (Fig. 2). In addition, a lesser amount of lysis was noted on both H-2L\textsuperscript{d}, and Q10\textsuperscript{d}/L\textsuperscript{d}-expressing targets. This weaker lysis is consistent with a subset of the B6 anti-H-2K\textsuperscript{bm1} CTL population being crossreactive. Thus, within the repertoire of B6 anti-H-2K\textsuperscript{bm1} CTL there exist populations that recognize H-2L\textsuperscript{d}, Q10\textsuperscript{d}/L\textsuperscript{d}, and another H-2\textsuperscript{d}-encoded MHC molecule, possibly H-2K\textsuperscript{d}.

Anti-Q10 CTL Recognition of H-2K\textsuperscript{bm1} is Affected by Mutations at Both Positions 152 and 155-156. Since the H-2K\textsuperscript{bm1} mutation is a complex event involving three amino acid substitutions, it is not possible to distinguish which of these changes controls the immunodominant epitope recognized by anti-Q10 CTL. To address this, anti-Q10 CTL were tested for their ability to recognize H-2K\textsuperscript{bm1} molecules in which amino acid position 152 or 155 and 156 were changed back to the wild-type H-2K\textsuperscript{b} residue by site-directed mutagenesis. The data in Fig. 3 indicate that the presence
of the H-2K\textsuperscript{b} residue glutamic acid rather than alanine at position 152 eliminates recognition of H-2K\textsuperscript{bm1} by anti-Q10\textsuperscript{d} CTL. Similarly, the presence of H-2K\textsuperscript{b} residues arginine and leucine rather than tyrosine at positions 155 and 156 also results in a loss of recognition. It should be noted that both revertant cell lines express equivalent amounts of antigen as that of H-2K\textsuperscript{b} and H-2K\textsuperscript{bm1}-expressing L cell lines, as determined by RIA (32) and cytofluorimetry (data not shown). In addition, both revertant cell lines are lysed by B6 anti-H-2K\textsuperscript{bm1} CTL (32). The background lysis seen on H-2K\textsuperscript{b} is a phenomenon observed by us and other investigators.
and is especially noted when effector cells are taken from animals previously primed in vivo with L cell transfectants (29, 30, 42). This lytic activity is not specific for H-2Kb since equivalent lysis is also detected on L cells transfected with the tk gene only (data not shown). Thus, the recognition of H-2Kbm1 by anti-Q10 CTL requires the mutant amino acids at both positions 152 and 155-156.

Animals Tolerant to H-2Kbm1 do not Generate anti-Q10 CTL that Crossreact on H-2d Antigens. The previous data indicate that anti-Q10 CTL crossreact on H-2Kbm1. We have previously shown that these CTL recognize H-2Kd, H-2Ld, and a third H-2d-encoded molecule controlled by a gene mapping between H-2Dd and H-2V (30). To further establish the existence of a common epitope in multiple class I alloantigens recognized by anti-Q10 CTL, CTL were generated using responder spleen cells from (C3H × H-2Kbm1)F1 mice. We reasoned that only a portion of the anti-Q10 CTL response would be directed at the common epitope controlled by the amino acids at positions 152, 155, and 156. Thus, by removing the subset of CTL reactive against this epitope (by using mice expected to be tolerant to this determinant), a loss of crossreactivity against other molecules containing the postulated common epitope should occur. The data in Fig. 4 show that (C3H × H-2Kbm1)F1 mice generate anti-Q10-specific CTL activity, as evidenced by lysis of Q10d/Ld L cell targets (left panel). However, reactivity on H-2Kd-expressing B10.D2(R103) (K2Dd) and H-2Ld-expressing B10.A(3R) (K2DdLd) target cells does not occur (right panel). In addition, H-2Kbm1 targets are not lysed (data not shown). Thus, tolerance to H-2Kbm1 results in a loss of reactivity to the other H-2d-encoded crossreactive antigens.

In a reciprocal fashion, anti-Q10 CTL were generated using spleen cells from (C3H × BALB/c)F1 responders. The data in Fig. 5 show these mice capable of generating an anti-Q10-specific response by killing Q10d/Ld L cell targets (left panel). However, reactivity on H-2Kbm1 target cells is greatly reduced (right panel). Thus, these data support the postulate that H-2d molecules, H-2Kbm1, and Q10d share a common epitope defined in part by amino acids 152, 155, and 156.
We further tested whether (C3H × BALB/c-H-2²dm²)F₁ mice could generate anti-Q10 CTL that crossreact on H-2Ld and H-2Kbm¹. While BALB/c-H-2²dm² (K'dD') mice have deleted the H-2Ld gene (43), they do express H-2Kd that shares tyrosines at positions 155 and 156 with Q10. The data in Fig. 6 indicate that cells from these mice generate strong lytic activity on H-2Kbm¹ and H-2Ld-expressing B10.A(3R) targets (right panel). Since H-2Kd differs from H-2Ld and H-2Kbm¹ at position 152 in that H-2Kd encodes an aspartic acid rather than an alanine, this suggests that this residue has a significant effect on the recognition of the crossreactive epitope. However, it should also be noted that all three of these molecules have other amino acid differences, particularly in the a1 domain, as will be discussed later.

Specificity of Anti-Q10 CTL Clones. Although the data presented indicate that the presence of certain amino acids at positions 152, 155, and 156 coincides with Q10 crossreactivity, it is not clear whether the same CTL receptor interacts with each alloantigen. Thus, it is possible that different CTL subsets are involved in the recognition of each molecule. To resolve this, anti-Q10d bulk CTL cultures were cloned by limiting dilution on H-2Kbm¹ or BALB/c feeder cells. This approach was necessary since attempts to clone and maintain long-term lines using L cell stimulators were unsuccessful.

The lytic activity of the clones generated were tested on a panel of target cells and grouped into four patterns of reactivity. The data in Fig. 7 indicate that group I clones show significant lysis of H-2Kbm¹ and Q10d/Ld-transfected L cells, but only marginal recognition of H-2Ld. Group II clones exhibit reactivity on H-2Ld and Q10d/Ld, but do not lyse H-2Kbm¹ targets. Group III clones exhibit reactivity on H-2Kbm¹, H-2Ld, and Q10d/Ld-expressing targets. Of particular interest is the reactivity of group IV clones. These clones have strong reactivity on L cells expressing H-2Kbm¹, H-2Ld, or Q10d/Ld molecules, and weak reactivity on H-2Dd. In addition, they recognize H-2Kd since they lyse B10.D2(R103) (K'dD') but not B10.
Anti-Q10 CTL clones demonstrate multiple crossreactivity patterns. (C3H x B6.K1)F1 anti-Q10 CTL clones were maintained on H-2K\textsuperscript{blm1} or H-2\textsuperscript{d} stimulator cells. Specific release of \textsuperscript{51}Cr from Q10/Ld, H-2K\textsuperscript{blm1}, and H-2D\textsuperscript{d} transfected L cells is indicated. Effector/target ratio is 25:1. Each group consists of multiple anti-Q10 CTL clones with the same reactivity pattern. A representative from each group is shown in the figure.

Changes in the Sequence of the a1 Helix between Positions 63 and 73 of H-2L\textsuperscript{d} Affects Recognition by anti-Q10 CTL Clones. The data thus far describe a CTL epitope controlled by amino acids 152, 155, and 156 in the a2 domain. This epitope can be recognized by bulk and cloned anti-Q10 CTL and is present in multiple class I
The panel of anti-Q10 CTL clones were tested for their ability to recognize three independent H-2L^d mutant molecules. M66 acquired H-2D^d amino acids at positions 63, 65, and 66; M70 acquired an additional H-2D^d amino acid at position 70; and M73 included H-2D^d-specific changes at positions 63, 65, 66, 70, and 73 (Table II). The effect of these various amino acid substitutions on the recognition of H-2L^d by anti-Q10 CTL correlates with the crossreactivity patterns of the various groups of anti-Q10 CTL clones. Thus, group I clones display marginal lysis of wild-type H-2L^d cells and no lysis of M66 or H-2D^d-expressing L cell targets (Fig. 8). However, this group does crossreact on the mutants M70 and M73. H-2K^bm1, Q10^d, and H-2D^d have identical amino acids at positions 63, 70, and 73, and at position 66, Q10^d and H-2D^d have an arginine while H-2K^bm1 has a lysine (Table II). Thus, introducing changes into the H-2L^d molecule to produce M70 and M73 makes H-2L^d more homologous to H-2K^bm1 and Q10^d and allows H-2L^d to gain the crossreactive epitope. CTL clones from groups II and III, which react on H-2L^d and Q10^d/L^d, do not react on the M66 mutant cell line. However, they show strong reactivity on the M70 and M73 mutant cell lines. This suggests that the introduction of the changes at positions 63, 65, and 66 causes a loss of the H-2L^d (a2 domain–controlled) epitope that can be restored by an additional amino acid substitution at position 70. The additional change at position 73 has no discernable effect. Group IV CTL clones, which crossreact on H-2K^bm1, H-2L^d, H-2D^d, and Q10^d/L^d, show strong reactivity on M66, M70, and M73 cell lines. Thus, the recognition of H-2L^d by this group is not influenced by H-2D^d-specific amino acid substitutions in the a1 domain. These clones do, however, exhibit weak activity on H-2D^d, as previously noted. Taken together, these data show that while amino acids at posi-
## Table II

### a1 Domain Amino Acids Involved in Binding Antigenic Peptides and/or the TCR

| Molecule | a1 Strands | a1 Helix |
|----------|------------|----------|
| Q10      | Met Tyr Glu Phe Iso Gly Pro Glu Tyr Glu Arg Glu Thr Gln Arg Ala Lys Gly Asn Gln Glu Ser Phe His Val Ser Thr Leu Leu His |
| H-2K<sup>b</sup> | Leu Val Tyr Glu | Arg Asp Tyr |
| H-2K<sup>bml</sup> | Leu Val Tyr Glu | Arg Asp Tyr |
| Glu(152) | Leu Val Tyr Glu | Arg Asp Tyr |
| Arg-Leu | Leu Val Tyr Glu | Arg Asp Tyr |
| (155–156) | | |
| H-2L<sup>d</sup> | Tyr Ser Iso Iso Gln Trp Arg Asn Tyr |
| M66 | Tyr Ser Arg Gln Trp Asn Tyr |
| M70 | Tyr Ser Arg Trp Asn Tyr |
| M73 | Tyr Ser Arg Trp Asn Tyr |
| H-2D<sup>d</sup> | Leu Val Tyr Glu Arg Asp Ala Tyr |
| H-2K<sup>d</sup> | Leu Val Ala Glu Gln Ser Asp Trp Arg Ala Gln Tyr |

Amino acid sequence data for the Q10, H-2K<sup>b</sup>, H-2L<sup>d</sup>, H-2D<sup>d</sup>, and H-2K<sup>d</sup> molecules. Sequences for the genetically engineered Glu(152), Arg-Leu(155–156) and M66, M70, and M73 molecules are from references 52 and 53. Underlined residues indicate residues with a variability index >6 (45). The residues listed have been postulated by Bjorkman et al. (21) to contact the TCR and/or antigenic peptides.
Figure 8. Anti-Q10 CTL recognition of H-2Ld is differentially affected by α1 domain substitutions. (C3H × B6.K1)F1 anti-Q10 CTL clones were maintained on H-2Kbm1 or H-2d stimulator cells. Specific release of 3HCr from α1, H-2Ld, M66, M70, and M73 transfected L cells is indicated. Effector/target ratio is 25:1. Each group consists of multiple anti-Q10 CTL clones with the same reactivity pattern. A representative of each group is shown in the figure.

152, 155, and 156 control the expression of the Q10 crossreactive epitope, other amino acids in the α1 helix also influence this determinant.

Discussion

We have shown that bulk-cultured CTL generated against the hybrid class I molecule Q10d/Ld crossreact on H-2Kbm1, H-2Ld, and H-2Kd. This finding was confirmed by demonstrating that CTL clones derived from these bulk cultures displayed a similar specificity. Since H-2Kbm1 revertant molecules that have amino acids at positions 152 or 155 and 156 changed back to the wild-type H-2Kb residues are not recognized by these CTL, this reactivity coincides with the presence of an alanine or aspartic acid (in the case of H-2Kd) at position 152 and tyrosines at positions 155 and 156 in the α2 helical region in all four of these molecules. Support for the presence of this common epitope also comes from the demonstration both by us and others that anti-H-2Kbm1 CTL crossreact on H-2Ld, Q10d/Ld, and H-2Kd and the finding that anti-H-2Ld CTL recognize both Q10d/Ld and H-2Kbm1 (19, 29, 40, 41).
The three-dimensional structure of the human class I molecule HLA-A2 has been recently shown to have a surface consisting of two antiparallel α-helical regions overlaying eight β-pleated strands. It has been suggested that residues in the helices, together with particular amino acids on the strands, are involved in binding antigenic peptides and/or the TCR receptor (20, 21). The α2 domain-encoded helix consists of amino acid residues 138-173, which includes positions 152, 155, and 156. Some of the CTL-defined HLA class I variants have amino acid substitutions in this region (16, 17). HLA-A3.2 varies from the HLA-A3.1 molecule at positions 152 and 156 and the substitution at residue 152 has been shown to profoundly affect how these molecules are recognized by both alloreactive and MHC-restricted CTL (44). Thus, in both mouse and man substitutions involving residues 152, 155, and 156 affect particular CTL epitopes. To further underscore the role of these amino acids in controlling CTL determinants, it should be noted that the H-2 class I molecules containing the Q10 crossreactive epitope vary considerably. For example, Q10 differs from H-2Kbm1, H-2Ld, and H-2Kd, by 26, 25, and 39%, respectively, of amino acids contained in the two helices and β strands. These amino acids represent residues that could potentially contact an antigenic peptide or TCR (21) (see Tables II and III). Further, approximately half of these differences represent amino acids with a variability index greater than six (45) (see Tables II and III).

Polymorphic determinants recognized by alloreactive CTL have been mapped to the α1 and α2 external domains of class I molecules (22, 46, 47). H-2 antigens consisting of α1 and α2 domains derived from different class I molecules have been created in an attempt to determine whether CTL determinants are linear structures in either α1 or α2, or nonlinear dependent on amino acids from both domains. When this was studied using H-2Dd and H-2Ld domain-shuffled molecules, it was noted that anti-H-2Dd and anti-H-2Ld CTL recognized a construct consisting of Dd amino acids in the α1 and Ld amino acids in the α2 domain (26). This finding suggests that H-2Dspecific epitopes are primarily controlled by the α1 domain; whereas H-2Ld epitopes, including the Q10 crossreactive epitope described here, are controlled primarily by amino acids in the α2 domain. This is supported by the finding that the reciprocal Ld/Dd domain-shuffled molecule was recognized by neither anti-H-2Dd or anti-H-2Ld CTL (27). Recently, we further defined the importance of amino acid residues 63-73 in controlling H-2Dd CTL epitopes by showing that changing amino acids in this region of the H-2Ld molecule to the H-2Dd type allowed these mutant molecules to be recognized by anti-H-2Dd CTL (33).

H-2Ld epitopes have also been localized to the α2 domain by demonstrating that anti-H-2Ld CTL recognize the recombinant molecule H-2dm1 which contains Dd amino acids in the α1 and NH2-terminal end of the α2 domain and Ld amino acids in the COOH-terminal end of α2 (48, 49). We have noted that anti-Q10 CTL have a similar reactivity against H-2dm1 (30).

Since most other domain-shuffled class I molecules are not recognized by alloreactive CTL (22-25, 27), it is likely in the above-mentioned examples that α1 domain H-2d amino acids are permissive for the expression of H-2Ld α2 domain epitopes and vice versa. The data presented in this study not only support this possibility but identify specific amino acids in the α1 helix that control the expression of the Q10 crossreactive epitope. For example, although group I anti-Q10 clones marginally recognize H-2Ld, they do react with H-2Ld mutant molecules that have H-2Dd...
### Table III

**α2 Domain Amino Acids Involved in Binding Antigenic Peptides and/or the TCR**

| Molecule | 93 | 97 | 99 | 114 | 116 | 143 | 145 | 146 | 147 | 149 | 150 | 151 | 152 | 154 | 155 | 156 | 157 | 158 | 159 | 161 | 162 | 163 | 165 | 166 | 167 | 169 | 171 |
|----------|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Q10      |    |    |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| H-2Kb    | Val Ser Gln  | His  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| H-2Kbm   | Val Ser Gln  | His  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Glu(152) | Val Ser Gln  | His  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Arg-Leu  | Val Ser Gln  | His  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

(155–156)

| H-2Ld    | Leu  | Glu  | Phe  |    |    |    |    |    |    |    |    |    |    |    | Gly |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| H-2Ld    | Leu  | Glu  | Phe  |    |    |    |    |    |    |    |    |    |    |    | Gly |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| M66      | Leu  | Glu  | Phe  |    |    |    |    |    |    |    |    |    |    |    | Gly |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| M70      | Leu  | Glu  | Phe  |    |    |    |    |    |    |    |    |    |    |    | Gly |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| M73      | Leu  | Glu  | Phe  |    |    |    |    |    |    |    |    |    |    |    | Gly |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| H-2Dd    | Leu  | Ala  | Trp  | Phe | Tyr |    |    |    |    |    |    |    |    |    | Arg | Asp | Gly |    |    |    |    |    |    |    |    |    |    |    |    |    |
| H-2Kd    | Phe  | Arg  | Phe  | Gln | Phe | Tyr |    |    |    |    |    |    |    |    | Asp |    |    |    | Gly |    |    |    |    |    |    |    |    |    |    |

See Table II for explanation. Residues 152 and 155 of the HLA-A2 molecule have 43% and 61% of their surface accessible to solvent (P. Bjorkman, personal communication). The spatial orientation of Q10 residues in this α-helical region is similar to those in the HLA-A2 molecule (P. Bjorkman, personal communication). Thus, it is possible that residues 152 and 155 contact the TCR (see text for explanation).
amino acid substitutions at position 63, 65, 66, and 70 of the α1 domain. Groups II and III clones, which crossreact on H-2Ld, were negatively influenced by substitutions in the α1 domain with a resulting loss of H-2Ld recognition. Thus, while CTL epitopes may appear to be mapped solely to amino acids present in the α1 or α2 domain-encoded α helices, the data presented in this report demonstrate that α2 domain epitopes are dramatically affected by alteration of amino acids in the α1 domain.

Using computer-aided modeling, the spatial positioning of Q10 residues relative to HLA-A2 was found to be quite similar (P. Bjorkman, personal communication). However, there is an effect on the size of the proposed antigen-binding cleft between the two helices in that the tyrosine residues at positions 155 and 156 in Q10 have significantly larger side chains than the glutamine and leucine residues present in the HLA-A2 molecule. Since these residues point down and into the cleft in both Q10 and HLA-A2, the tyrosines in Q10 reduce the size of the pocket in this region. Further, amino acids at positions 97 (tryptophan), 99 (tyrosine), 159 (tyrosine), and 167 (tryptophan) all have large side chains, and due to their orientation in the groove, also tend to reduce its size (see Table III). It is interesting to note that unlike H-2Kb, the H-2Kbm1 molecule (which has tyrosines at positions 155 and 156) does not function as a restricting element for most antigens that have been tested (50–52). It is possible that H-2Kbm1 with a reduced groove size can no longer bind antigenic peptides, especially if they require an α-helical conformation, as previously suggested (53–55). Alternatively, the size of the Q10 pocket may limit the number of peptides capable of binding to this class I molecule or allow for peptides to bind with a linear rather than α-helical configuration.

It has been proposed that alloreactive CTL may recognize class I molecules together with self peptides that occupy these pockets (21). If the Q10 molecule has a smaller peptide binding cleft, then it is possible that a relatively large proportion of cell membrane Q10/Ld molecules lack self peptides. This would tend to make CTL recognition of Q10 specific for amino acids on the two helices, directly. Thus, this may explain the strong influence of the residue at position 155, whose side chain is oriented in the presumed direction of the TCR, and residue 152, which should have a similar orientation on the α helix, in controlling the crossreactive epitope.

Summary

CTL derived from (C3H × B6.K1)F1 animals were sensitized against L cells that express the transfected gene product Q10d/Ld. These CTL were highly crossreactive against three other class I molecules, H-2Kbm1, H-2Ld, and H-2Kd. In an attempt to define this crossreactive epitope it was noted that between 25 and 39% of amino acids in the α helices and central β strands of these three molecules vary from Q10d. These amino acids represent residues that have been proposed to potentially interact with a peptide antigen or TCR (21). However, all four molecules share the amino acid tyrosine at positions 155 and 156. Additionally, Q10d, H-2Kbm1, and H-2Ld share alanine at position 152, while H-2Kd has an aspartic acid. We showed that these residues were important in controlling this epitope by the finding that anti-Q10d CTL did not recognize H-2Kbm1 revertant molecules that had either the position 152 alanine changed back to the wild-type H-2Kb residue (glutamic acid) or position 155 and 156 tyrosines changed back to wild-type residues arginine and
leucine. Further evidence that these molecules share a crossreactive epitope was noted by the failure of (C3H × H-2Kbnm1)F1 animals to generate CTL that recognized H-2Ld or H-2Kb, and the inability of (C3H × BALB/c)F1 animals to generate CTL reactive against H-2Kbnm1. CTL from these mice were still able to recognize Q10d/Ld, indicating that other epitopes could be detected if natural tolerance prevented recognition of the crossreactive epitope. To further define the epitope, CTL clones were generated against Q10d/Ld and maintained on either H-2Kb or BALB/c feeder cells. In addition to testing these clones on the target cells described above, mutant molecules derived from H-2Ld, which have amino acid substitutions in their α1 domain, were analyzed. It was noted that some anti-Q10 clones that did not crossreact on H-2Ld did react against H-2Ld mutant antigens that had H-2Dd amino acid substitutions in the α1 domain at positions 63, 65, 66, and 70. Other clones had differential reactivities on these H-2Ld mutants further substantiating that α1 domain amino acids play a role in controlling the expression of the crossreactive epitope.

Thus, four class I molecules with multiple amino acid differences in their α1 and α2 domains share a crossreactive epitope readily recognized by alloreactive CTL. This epitope is dependent on the presence of amino acids at positions 152, 155, and 156 in the α2 helix as well as amino acids between residues 63 and 73 in the α1 helix. The role of these residues in defining the nature of this alloreactive epitope is discussed.

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