MUTATIONS IN THE α2 HELIX OF HLA-A2 AFFECT PRESENTATION BUT DO NOT INHIBIT BINDING OF INFLUENZA VIRUS MATRIX PEPTIDE

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Knowledge of the mechanism by which class I-restricted CTL recognize target cells has been significantly advanced by two recent findings. First, Townsend et al. (1, 2) demonstrated that target cells that had been incubated with short synthetic peptides corresponding to sequences of the influenza nucleoprotein (NP)[1] molecule could be specifically recognized by class I-restricted, NP-specific CTL. These initial findings were subsequently extended to include CTL recognition of a number of peptides derived from viral molecules (3, 4) and alloantigens (5–7). These observations suggested that class I molecules bind and present peptide fragments to CTL by a mechanism that is as yet indistinguishable from that by which class II molecules present peptide antigens to Th (8–10). Secondly, Bjorkman et al. (11, 12) have resolved the protein structure of the human class I molecule HLA-A2 by x-ray crystallography. In this structure, the α1 and α2 domains interact to form a platform consisting of a β-pleated sheet floor over which lie two α helices with a long groove between them. The location, size, and shape of this groove is consistent with its proposed function as a peptide binding site. In fact, an electron-dense region (possibly a peptide) of unknown origin was found in this putative peptide binding site in the HLA-A2 crystal. Thus, it was suggested that class I molecules can bind peptides and present them to T cells in an analogous way that class II molecules have been shown to bind and present antigen (12).

To further analyze the role of class I molecules in the presentation of peptide antigens, we have examined the ability of peptide-specific CTL to recognize site-directed mutants of HLA-A2. A naturally occurring variant of A2.1 (DK1-A2.3) was previously identified by its inability to be recognized by A2.1-restricted influenza-specific CTL (13, 14). Characterization of the A2.3 gene (15, 16) has shown that these two

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[1] *Abbreviation used in this paper:* NP, nucleoprotein.

molecules differ at amino acid residues 149, 152, and 156. The side chains of amino acids at positions 152 and 156 point into the putative peptide binding site, whereas the side chain of the amino acid at position 149 points up and away from the site (11, 12). In this study, we have examined whether these substitutions affect the ability of the A2 molecule to present peptide to A2.1-restricted CTL. To do this, we have taken advantage of the finding that the major component of the A2.1-restricted CTL response to the type A influenza virus A/JAP/57 is the recognition of a single matrix protein peptide, M1 55–73 (3). Using human target cells transfected with site-directed mutants of the A2.1 gene, we have analyzed the effects of substitutions at positions 149, 152, and 156 on the ability of the A2 molecule to present this M1 peptide to peptide-specific CTL.

Materials and Methods

Cells. PBL were obtained by batch leukapheresis of normal adult volunteers (13). HLA serotyping of PBL was kindly performed by the Human Leukocyte Antigen Typing Lab, Department of Transfusion Medicine, National Institutes of Health, Bethesda, MD. JY and M16B are Epstein-Barr virus-transformed B cell lines.

Mutagenesis and Transfection. Site-directed mutagenesis was performed as described by Kunkel (17) and will be described in more detail elsewhere (Hogan et al., manuscript in preparation). Briefly, a 212-bp KpnI fragment encoding amino acids 113–182 of the HLA-A2.1 gene was subcloned into the vector M13mpl9. Oligonucleotides were synthesized such that they contained a mismatched nucleotide at the site to be mutated and were annealed to the single-stranded template DNA. A complete second strand was then synthesized by in vitro primer extension. After in vivo selection of phage containing mutant DNA (17), the mutants were confirmed and identified by Sanger dideoxy sequencing (18). KpnI inserts containing multiple mutations were generated by additional cycles of mutagenesis with the appropriate oligonucleotides. The KpnI fragment was then reinserted into a plasmid vector containing the complete A2.1 gene, but with the corresponding 212-bp KpnI fragment deleted. Plasmids containing the inserted fragment in the correct orientation were identified by restriction mapping. To ensure that the proper fragment had been subcloned, the same fragment was removed from the putative mutant DNA, subcloned into M13mpl8 or M13mpl9 and resequenced. The A2 mutants used in this study are described in Table I. The nomenclature used is the single letter code for the amino acids at positions 149, 152, and 156. Underlined residues indicate those amino acids that are different from those found in A2.1.

The cell line used for transfections was HMy2.C1R (HMy), a class I^- deletion mutant of the human plasma cell leukemia line LICR.LON.HMy2 (19) (kindly provided by Dr. Peter Cresswell, Duke University, Durham, NC). An Eco RI/Bam HI fragment containing the neomycin resistance gene was subcloned from pKOneo into each of the mutant genes. DNA (20–40 μg) was transfected into 5×10^6 HMy cells by electroporation (20). Cells were selected in culture with 600 μg/ml geneticin G418 (Gibco Laboratories, Grand Island, NY). Transfectants expressing high levels of mutant molecules were identified by RIA and FACS analysis. High expressors were then subcloned by limiting dilution to ensure the clonality of the cell population. The cells were periodically screened to ensure stable expression of the mutant gene products.

FACS Analysis of Transfectants. Cell surface expression of transfected A2 genes was assessed by indirect immunofluorescence using a FACS IV (Becton Dickinson & Co., Sunnyvale, CA). Cells were incubated with a saturating amount of the A2-specific mAb BB7.2 (21, a 1:1,000 dilution of ascites), washed, and then incubated with fluorescein-labeled anti-mouse Ig (Cappel Laboratories, West Chester, PA) as previously described (22). The results of these assays are shown in Fig. 1. All transfectants expressed similar levels of cell surface A2.

Synthetic Peptides. Synthetic peptides that correspond to the sequences 55–73 (LTKGILGFVFTLTVPSERG) and 57–68 of the M1 protein (3) and 181–204 (INETEQRTLYENVGTYVSVGTSTL) of the hemagglutinin (HA) of A/JAP influenza virus (23) were

TABLE I
Class I Genes Used for Transfection

Gene	Amino acid position		
	149	152	156
A2.1 = AVL	Ala (GCG)	Val (GTG)	Leu (TTG)
A2.3 = TEW	Thr (ACG)	Glu (GAG)	Trp (TGG)
TVL	Thr (ACG)	Val	Leu
AEL	Ala	Glu (GAG)	Leu
AVW	Ala	Val	Trp (TGG)
TVW	Thr (ACG)	Val	Trp (TGG)
AAL	Ala	Ala (GCG)	Leu

DNA and amino acid sequences of A2.1, A2.3, and the site-directed mutants of A2.1. The nomenclature of the mutants is such that each of the three letters corresponds to the single letter amino acid code for positions 149, 152, and 156. Underlined residues indicate a substitution relative to A2.1.

synthesized by automatic solid phase synthesis on a peptide synthesizer (model 430A; Applied Biosystems, Inc., Foster City, CA) or were purchased from Peninsula Labs (South San Francisco, CA). M1 peptides were extracted with aqueous volatile buffer, lyophilized to dryness, and used without further purification (24).

Generation and Assay of CTL. PBL from A2.1-positive individuals (3×10^6) were cultured in vitro with 5 μg/ml M1 57–68 peptide (~3.4 μM) for 9 d in 2 ml of culture medium [RPMI 1640 (Gibco Laboratories) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100

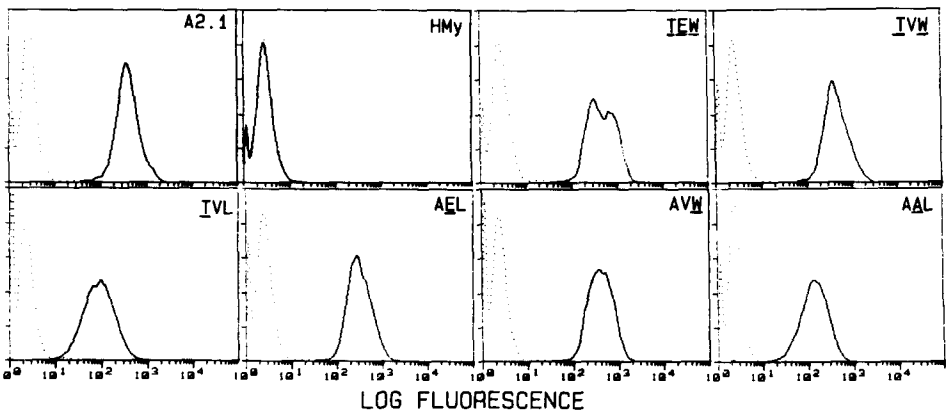

FIGURE 1. FACS analysis of HMy transfected cells. Untransfected HMy and transfected cells were analyzed with A2-specific mAb BB7.2 by indirect immunofluorescence. Solid lines are profiles of cells stained with BB7.2 plus Fl-anti-Ig, and dotted lines are profiles of cells stained with Fl-anti-Ig alone. The amino acids at positions 149, 152, and 156 are used to identify each of the transfected genes. Underlined residues designate amino acids that are different from those found in the A2.1 gene.

μg/ml streptomycin, and 10% pooled heparinized human plasma] in 24-well tissue culture plates (Nunc, Roskilde, Denmark). 5 U/ml rIL2 (Cellular Products, Buffalo, NY) were added on day 3 of culture. On day 10, the primed responder cells were restimulated in secondary culture: 10^6 responder cells and 9×10^6 irradiated autologous PBL (2,000 rad) were cultured in the presence of 5 μg/ml M1 57–68 peptide and 5 U/ml rIL-2 for 5 d in 10 ml of culture medium in upright 25-cm^2 flasks (Nunc). Tertiary and quaternary stimulations of responder cells were done exactly as described for the secondary stimulation. After secondary stimulation, responder cells were plated in limiting dilution culture (25) at 10 cells per well or 1 cell per well in the presence of 5 μg/ml M1 peptide 57–68, 25 U/ml rIL-2, and irradiated A2.1-positive PBL (2,000 rad).

The generation of the A2-specific alloreactive CTL line B12.A2.C5 has been described (26). The HLA-DPw4-specific CTL line H9 anti-W7 (27) was kindly provided by Dr. Stephen Shaw, NIH, Bethesda, MD.

CTL activity was measured in a standard 4–5-h ^{51}Cr-release assay (22). For preparation of targets with the M1 peptide, cells were incubated for 18 h (unless otherwise stated) with 100 μCi of $Na_2{}^{51}CrO_4$ (New England Nuclear, Boston, MA) and an optimal concentration (5 μg/ml, ~2.2 μM) of M1 55–73 peptide (in preliminary experiments, this peptide was shown to be slightly better for sensitizing targets than the 57–68 peptide). Targets were then washed twice, counted, and plated in the presence of 10 μg/ml M1 55–73 peptide for the duration of the CTL assay. In some cases, targets were also infected with A/JAP/57 or B/Ann Arbor influenza viruses (22) rather than being pulsed with M1 peptides. Results are expressed as the mean percent specific lysis of triplicate determinations (22).

Results

Generation and Specificity of HLA-A2.1-restricted M1 Peptide-specific CTL. PBL from A2.1-positive individuals were stimulated in primary in vitro culture with 5 μg/ml M1 57–68 peptide in the presence or absence of 5 U/ml rIL-2. CTL activity was assessed on A2-matched JY target cells incubated with or without M1 55–73 peptide. The results of one experiment with three different individuals are shown in Fig. 2. CTL activity could be detected in PBL populations stimulated with peptide plus rIL-2, but little or no activity was generated in the absence of rIL-2. No cytolytic activity was detected on target cells that had been incubated without peptide. M1 peptide-primed cells restimulated in secondary, tertiary, and quaternary cultures gave a similar reactivity pattern (data not shown).

CTL lines generated in limiting dilution culture were established from these bulk restimulations. The antigen specificity of a representative CTL line is shown in Fig. 3. This CTL line specifically lysed A2.1-matched targets (JY) incubated with the M1 55–73 peptide but did not lyse the same targets incubated with an unrelated antigenic A/JAP peptide HA 181–204. The A2.1-negative target M16B that was incubated with the M1 peptide was not lysed. JY targets infected with A/JAP influenza virus, from which the M1 55–73 sequence was derived, were lysed, whereas JY targets infected with B/Ann Arbor influenza virus were not lysed. These results demonstrate that M1 peptide, in the presence of rIL-2, can stimulate CTL that are able to specifically recognize A/JAP-infected A2-positive targets, as well as targets sensitized with the M1 55–73 peptide.

M1-Peptide-specific CTL Recognition of A2 Transfectants. A2.1-restricted CTL specific for the M1 peptide 57–68 were generated in secondary and quaternary bulk cultures and in limiting dilution culture. These CTL populations were assayed on the series of HMy transfectants described in the Materials and Methods section and shown in Fig. 1. The transfectants expressed A2.1 or site-directed mutants of the A2.1 gene

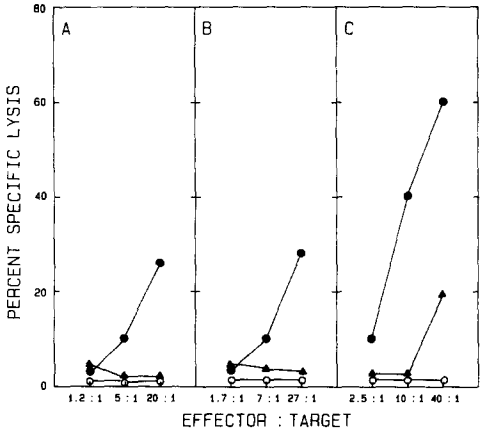

FIGURE 2. Generation of influenza virus M1 peptide-specific CTL. A2.1$^+$ PBL from three different individuals; Q53, (*A*); Q63, (*B*); and Q82, (*C*) were stimulated in 7-d primary cultures with peptide M1 57-68 with (●) or without (▲) rIL2. Effector populations were assayed on A2.1$^+$ target JY that was incubated with 10 μg/ml M1 55-73 (●, ▲) or no peptide (o).

that encoded molecules that differed by amino acids at positions 149, 152, and/or 156 (see Table I). The results shown in Fig. 4 (*A-C*) are representative of results obtained with 14 CTL lines generated in secondary or quaternary bulk cultures and 11 CTL lines generated in limiting dilution culture. All the CTL lines lysed A2.1 but not A2.3 (TEW) transfectants or untransfected cells that were incubated with M1 55-73 peptide. Mutants AEL and AVW, which expressed the A2.3 residues Glu at position 152 and Trp at position 156, respectively, were not lysed. The double mutant TVW, which expressed the A2.3 residues Thr at 149 and Trp at 156, also was not lysed. These results demonstrate that the introduction of single amino acid substitutions at positions 152 or 156 can dramatically alter the ability of the A2 molecule plus M1 55-73 peptide to be recognized by this group of CTL. In contrast, the presence of the A2.3-derived residue Thr at position 149 did not have a similar effect as the site-directed mutant TVL was lysed by all the CTL lines. In the absence

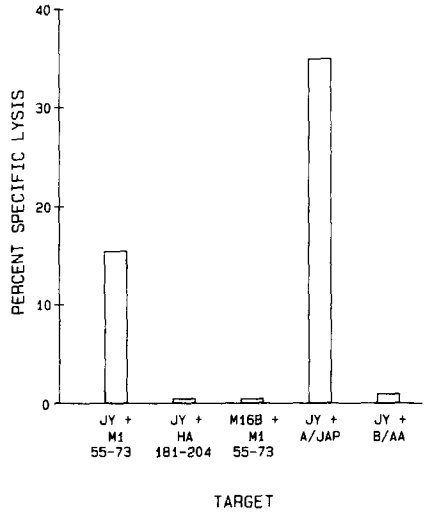

FIGURE 3. Specificity of M1 peptide-specific CTL. CTL line Q44.3B3 was generated against A2.1 plus M1 57-68 peptide in limiting dilution culture at 10 cells/well and assayed on A2.1$^+$ JY targets that were either incubated with M1 55-73 peptide, A/JAP influenza HA 181-204 peptide, or were infected with A/JAP or B/Ann Arbor virus. Targets also included A2.1$^-$ target M16B incubated with M1 55-73 peptide. E/T ratio, 28:1.

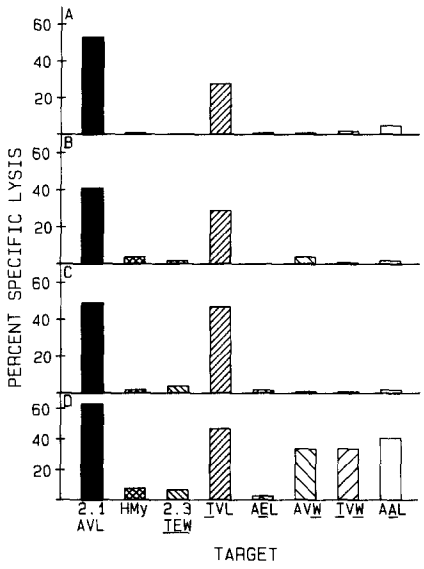

FIGURE 4. Recognition of HMy transfectants by A2.1 plus M1 55-73 peptide-specific CTL. CTL lines generated in secondary culture against M1 57-68 peptide (Q53, *A*, Q66, *D*), quaternary culture (Q44, *B*), and in limiting dilution culture at 10 cells/well (Q44.3B6, *C*) were assayed on the indicated panel of transfectants that had been incubated with 5 μg/ml of $M1_{55-73}$. E/T ratios were: (*A*, *B*, and *D*) 10:1; (*C*) 9:1. Underlined residues are those that are different from A2.1.

of peptide, no lysis of TVL was observed (data not shown). One mutant, AAL, was of particular interest because it contained an Ala for Val substitution at position 152. This substitution resulted in the loss of two methyl groups at this position, and is considered to be a relatively conservative substitution. The observation that the AAL mutant transfectant plus M1 peptide was not recognized by M1 55-73 peptide-specific A2.1-restricted CTL indicates that a conservative change at position 152 can have a major effect on recognition. In summary, amino acid changes that were made at positions 152 and 156 had dramatic effects on T cell recognition, whereas the change made at position 149 had little effect.

1 CTL line out of the 25 that were tested from nine different individuals was found to have a significantly different reactivity pattern with the site-directed mutants than was described above. Secondary CTL bulk cultures from donor Q66 (Fig. 4 *D*) lysed A2.1 and TVL transfectants but not the A2.3 (TEW) or 152 mutant AEL transfectants or untransfected HMy cells plus M1 peptide, as was observed for the other

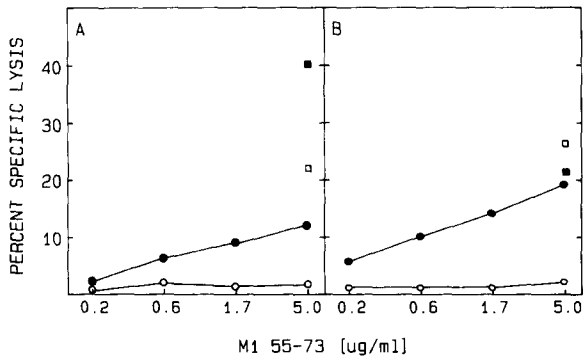

FIGURE 5. Effect of Thr substitution for Ala at position 149. A2.1-restricted M1 55-73 peptide-specific CTL clones Q44.IC5 (*A*) and Q44.3B6 (*B*) were assayed on 2.1 (AVL, ■) and TVL (□) transfectants (E/T ratio, 5:1) that were either preincubated for 18 h with 5 μg/ml M1 55-73 or 2.1 (AVL, ●) and TVL (○) transfectants that were not preincubated with peptide but were exposed to various doses of M1 55-73 during the 4-h CTL assay.

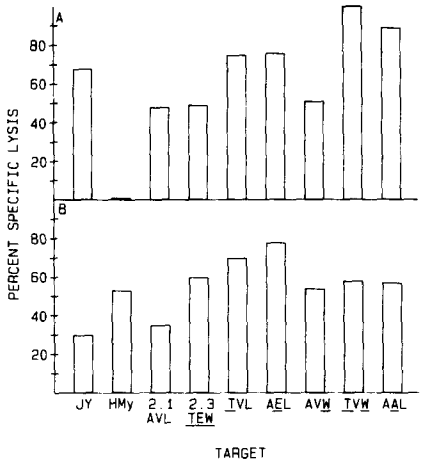

FIGURE 6. Susceptibility of transfectants to lysis by allospecific CTL. Transfectants were assayed as targets for anti-A2 allospecific CTL line B12.A2.C5 (*A*, E/T ratio, 2.5:1) and anti-DPw4-specific CTL line H9 anti-W7 (*B*, E/T ratio, 10:1).

CTL lines. In contrast, these CTL were able to lyse transfectants that express the single mutants AVW and AAL as well as the double mutant TVW. No lysis of these mutants was observed in the absence of M1 peptide (data not shown). These results indicate that A2 molecules with substitutions of either Thr for Ala at position 149, Ala for Val at 152, or Trp for Leu at position 156 can still present the M1 peptide to some T cells. Therefore, the lack of recognition of these mutants by the majority of peptide-specific A2.1-restricted CTL is not due to loss of peptide binding.

The observation that the mutant transfectant TVL was lysed by all CTL lines that were tested (Fig. 4) suggested that this substitution had little or no effect on recognition of the M1 55–73 peptide. In these experiments, targets were preincubated with peptide for 18 h before the CTL assay. However, significant differences were observed between the A2.1 and TVL transfectants when these targets were incubated with M1 55–73 peptide immediately before the CTL assay (Fig. 5). Both targets could be lysed by M1-specific CTL lines under normal assay conditions, but only the A2.1 transfectant was lysed when increasing amounts of the M1 55–73 peptide were added immediately before the CTL assay. These results indicate that the substitution of a Thr for an Ala residue at position 149 may affect the kinetics of binding of the M1 55–73 peptide to the A2 molecule. This substitution may also affect the binding affinity for peptide(s) that are already bound to the A2 molecule and must be displaced before the M1 55–73 peptide can associate.

Reactivity of Site-directed Mutants with Control CTL Populations. To determine whether the inability of some of the transfected cells (e.g., TEW, AEL) to be lysed by the A2.1-restricted M1 peptide-specific CTL was due to some general defect in their ability to be recognized by T cells, two different control CTL populations were examined. The first control population was an alloreactive A2-specific CTL line (B12.A2.C5) that recognizes both A2.1 and A2.3 (Fig. 6 *A*). This CTL line lysed the TEW and the AEL transfectants as well or better than the A2.1 transfectant. The TVL transfectant, which was lysed by all tested M1 peptide-specific CTL, was not significantly more susceptible to lysis than the other transfectants. Analysis of all the transfectants with a DPw4-specific CTL line (Fig. 6 *B*) indicated that all of these cells were

very similar in their susceptibility to lysis. Collectively, these results indicate that there are no obvious differences in the susceptibility of the target cells in this panel to CTL-mediated lysis.

Discussion

The results in this paper demonstrate that individual amino acid changes that occur between A2.1 and A2.3 have an effect on the ability of these molecules to present the M1 55–73 peptide to peptide-specific A2.1-restricted CTL. In particular, single amino acid substitutions that replace residues 152 or 156 of the A2.1 molecule with those found in A2.3 inhibit the ability of most peptide-specific A2.1-restricted CTL to recognize these molecules. In contrast, a single amino acid substitution at residue 149 had relatively minor effects on CTL recognition. Although no direct evidence has been presented that class I molecules can bind antigenic peptides, it has been assumed that such binding occurs and that the side chains of the amino acids that point into the putative binding site are involved in antigen presentation (11, 12). The amino acids at positions 152 and 156 are on the α helix of the α2 domain and are oriented such that their side chains point into the putative peptide binding site (11, 12). Both of these residues could be ligands for peptide binding or they could be important for binding by the TCR. Therefore, changes in the side chains of these amino acids could have several possible effects: (*a*) abolish the ability of the molecule to bind an antigenic peptide; (*b*) change the conformation of the peptide that is presented to the TCR; or (*c*) alter the part of the A2 molecule that directly contacts the TCR.

The effects of the substitutions of Val → Ala at 152 (A<u>A</u>L), Leu → Trp at 156 (AV<u>W</u>), and both Ala → Thr at 149 and Leu → Trp at 156 (<u>T</u>V<u>W</u>) could not be attributed to a failure of these mutant molecules to bind the M1 55–73 peptide, because one M1-specific CTL population was found that could recognize target cells that express these molecules after incubation with the peptide. Thus, the failure of these molecules to be recognized by the other M1-specific CTL lines may result from a change in the conformation of the M1 55–73 peptide that is presented to the TCR. Alternatively, these mutations may alter the ability of the TCR to directly interact with the A2 molecule.

The substitution at position 152 of Ala (with side chain of CH_3) for Val (with side chain of -CH-$[CH_3]_2$) resulted in the loss of recognition by all but one of the M1-specific CTL lines that were analyzed. Because this is a relatively conservative substitution, it seems more likely that this change would result in an alteration of the conformation of the bound peptide, or the position of the peptide in the binding pocket rather than having major effects on the ability of the TCR to bind to the complex of the M1 peptide with the A2 molecule. However, it is possible that the side chain of amino acid 152 provides an important contact point with the TCR in combination with an adjacent residue of the peptide. It was also observed that the A<u>E</u>L transfectant with the substitution of Val → Glu at position 152 was not recognized by any of the M1-specific CTL, including the Q66 CTL population. Although this substitution involves a charge difference, we have also obtained the same results using transfectants with a Gln substitution at position 152 (data not shown). Collectively, these results suggest that amino acid residue 152 is critically involved in either binding of the M1 55–73 peptide or interacting with the TCR. The

significance of the amino acid at position 152 for T cell recognition of class I molecules has also been shown by results of studies using site-directed mutants of HLA-A3 (26).

The substitution of the A2.3 residue Trp for the A2.1 residue Leu at position 156 had the same effect as the Ala for Val substitution at position 152: only one of the M1-specific CTL lines could recognize this mutant. Unlike the substitution of Ala for Val, which results in a slightly smaller but structurally similar side chain, the change at position 156 involves the substitution of the large indole ring of Trp for the branched aliphatic side chain of Leu. Despite the fact that this represents a major change in the size and structure of the side chain at this position, this mutant could still bind the M1 55–73 peptide. This may indicate that this residue does not play an important role in the binding of the peptide, or that its role depends upon the strong hydrophobic character of both Leu and Trp. Inclusion of an additional substitution of Ala → Thr at position 149 in the double mutant TVW did not destroy the ability of the molecule to be recognized by the Q66 CTL. Although obtained with a limited number of CTL, this result in conjunction with the findings using the mutant AEL, suggest that, of the amino acid residues that differ between A2.1 and A2.3, position 152 exerts the strongest effect on T cell recognition.

The side chain of the amino acid at position 149 points up and away from the proposed peptide binding site (11, 12). The substitution of Ala → Thr at position 149 has been shown to inhibit the binding of one A2/A28-specific mAb CR11-351 (28), suggesting that this residue is on the outer surface of the A2 molecule. This substitution did not abolish the ability of the molecule to be recognized by any of the M1-specific CTL lines, although in most lines, the level of lysis on the TVL transfectant was less than on the A2.1 transfectant. However, this substitution did have an effect on the ability of the M1 55–73 peptide to associate with the A2 molecule, since the TVL mutant transfectant (unlike the A2.1 transfectant) could not be recognized when the peptide was added immediately before the CTL assay. This indicates that this substitution may affect the kinetics with which the M1 55–73 peptide associates with the A2 molecule. This result also suggests either that residues that are apparently not directly in the peptide binding pocket (based on the crystal structure [11, 12]) may nonetheless contact part of the peptide or that they may produce indirect effects on other residues that are involved in peptide binding.

The present analysis of T cell recognition of the A2 molecule has used CTL that have been generated against a synthetic peptide, M1 55–73, that corresponds to a sequence of the influenza A/JAP/57 M1 protein. Nevertheless, these CTL apparently recognize a naturally-occurring antigen, since they are able to specifically lyse A/JAP/57-infected target cells. CTL that are specific for the M1 peptide have not been generated from individuals who are $A2^-$ (data not shown), indicating that this peptide can only be recognized by T cells that are restricted by A2.1 or other class I molecules that are very rare in the population. We have been unable to generate readily measurable CTL responses to the M1 peptide in the absence of rIL-2, presumably because this peptide is not recognized in the context of class II molecules and, therefore, does not induce a Th response. The observation that anti-M1 peptide CTL are readily generated from most $A2^+$ individuals that have been tested may reflect the fact that most adults have been multiply primed in vivo with cross-reactive type A viruses. Thus, the in vitro responses that we are generating are most likely derived from memory CTL.

Although no direct evidence has been presented that class I molecules can bind antigenic peptides, it has been assumed that such binding occurs and that the amino acid side chains that extend into the putative peptide binding site participate in this interaction (11, 12). The results of the present study demonstrate that changes in two residues in this site, 152 and 156, can affect presentation without abolishing binding. The ability to make substitutions in amino acids in the peptide binding site without loss of peptide binding suggests that there is a considerable degree of flexibility in the interaction between peptide and the class I molecule. Thus, the ability of a peptide antigen to stimulate class I-restricted CTL depends not only on the ability of the peptide to bind to the class I molecule, but also the manner in which it is presented.

Summary

Previous studies have suggested that MHC class I molecules bind and present peptides to CTL in a manner that is analogous to the presentation of peptides by class II molecules to Th. Crystallographic studies of HLA-A2 have led to the assignment of a putative peptide binding site that is bordered by two α helices consisting of residues 50–84 and 138–180. In this study, we have investigated whether residues in the α2 helix are involved in the binding and/or presentation of a peptide to CTL. We have generated CTL to type A influenza virus by stimulation of human PBL with a synthetic peptide from the influenza A virus matrix protein (M1 residues 57–68) in the presence of rIL-2. Such HLA-A2.1-restricted influenza virus-immune CTL do not recognize infected HLA-A2.3$^+$ targets. A2.1 and A2.3 differ by three amino acids in the α2 domain: Ala vs. Thr at position 149, Val vs. Glu at position 152, and Leu vs. Trp at position 156. Site-directed mutants of the A2.1 gene that encode A2 molecules that resemble A2.3 at positions 149, 152, and 156 have been constructed, transfected into human cells, and assayed for their ability to present the M1 peptide. The results demonstrate that most, but not all, A2.1-restricted M1-peptide-specific CTL fail to recognize M1 peptide-exposed transfectants with certain single amino acid substitutions at positions 152 and 156. In contrast, M1 peptide-exposed transfectants that express A2 molecules with an Ala → Thr substitution at position 149 were recognized by all CTL tested, but they exhibited an apparent difference in the kinetics of peptide binding. These results indicate that amino acid substitutions at positions 152 and 156 of the putative peptide binding site of the A2 molecule can affect presentation without eliminating binding, and indicate that the failure to recognize complexes between the peptide and the mutant A2 molecules is due to different TCR specificities and not to the failure to bind the peptide.

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