STRUCTURAL REQUIREMENTS FOR CLASS I MHC MOLECULE-MEDIATED ANTIGEN PRESENTATION AND CYTOTOXIC T CELL RECOGNITION OF AN IMMUNODOMINANT DETERMINANT OF THE HUMAN IMMUNODEFICIENCY VIRUS ENVELOPE PROTEIN

By HIDEMI TAKAHASHI,* RICHARD HOUGHTEN,‖ SCOTT D. PUTNEY,¶ DAVID H. MARGULIES,‡ BERNARD MOSS,§ RONALD N. GERMAIN,‡ AND JAY A. BERZOFSKY*

*From the *Molecular Immunogenetics and Vaccine Research Section, Metabolism Branch, National Cancer Institute; the ‡Laboratory of Immunology and §Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892; the ‖Department of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California 92307; and the ¶Repligen Corporation, Cambridge, Massachusetts 02139*

In cellular immunity, thymus-derived (T) lymphocytes recognize antigens in the context of cell surface glycoproteins encoded by MHC genes (1, 2). In general, T lymphocytes expressing the CD4 antigen on their surface recognize antigens in association with class II MHC molecules, such as I-A or I-E in mice and HLA-DR or -DQ in humans, whereas T lymphocytes bearing the CD8 molecule are restricted by class I MHC molecules, such as H-2K, -D, -L in mice and HLA-A, -B, -C in humans. For both T cell subsets, antigens are usually not seen in their native form but only as some denatured or degraded form of the protein actively processed by APCs (3, 4) and physically associated with class I or II MHC molecules (5, 6).

Recently much information about the detailed interaction between MHC molecules and antigenic peptides has been accumulated, especially for ligand pairs recognized by class II MHC molecule-restricted T helper cells (7, 8). However, detailed studies on which amino acid residues are involved in binding to class I MHC molecules for $CD8^+$ CTL recognition remain limited and have been carried out only in a few antigen systems (9–11). In this article we have analyzed the interaction between the class I D^d MHC molecle and a CTL peptide epitope composed of a 15-residue amino acid sequence (315–329: RIQRGPGRAFVTIGK) that we have identified in the HIV-1-IIIB gp160 envelope glycoprotein (12). We have examined the structural requirements of both D^d and of the peptide antigen for this interaction.

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Address correspondence to Dr. Jay A. Berzofsky, Building 10, Room 6B12, National Institutes of Health, Bethesda, MD 20892.

[1] *Abbreviations used in this paper:* CTLp, CTL precursors; CTM, complete T cell medium; MOI, multiplicity of infection.

First, we found that presentation of the epitope required both the specific α1 and α2 domains of the D^d molecule, based on studies using L cell transfectants expressing various recombinant or truncated class I molecules (13). Such an analysis with reciprocally shuffled class I MHC domains has not previously been performed for a defined antigenic peptide.

Second, the fact that this epitope derives from a naturally highly variable segment of the HIV-1 envelope, and is seen by human CTL (14) as well as murine CTL (12), afforded us the opportunity not only to study a class I MHC molecule–peptide interaction, but also to analyze the structural basis for the loss of T cell crossreactivity as the virus mutates. In particular, since the CTL specific for this site on the HIV-1-IIIB isolate do not crossreact with the HIV-1-RF variant isolate (15), we could use the natural HIV variant sequences to dissect the role of each amino acid in the peptide. We synthesized a series of peptides that differ at each of these positions individually to determine which substitutions affect the presentation of the peptide, and found that, surprisingly, only 322(R) and 324(F) appear to be the critical amino acids for D^d binding and presentation. In contrast, residue 325(V) appears to affect TCR interaction. Thus, a limited number of residues are critical for both interactions, and natural variation of the virus involves mutations in both the portion interacting with the TCR and the portion interacting with the MHC molecule. These latter results are also discussed in the context of viral escape from immune effectors, in view of the fact that this peptide is also an epitope for human HIV envelope-specific CTL (14).

Materials and Methods

Recombinant Vaccinia Virus. vSC-25 (recombinant vaccinia virus expressing the HIV-1-IIIB gp160 envelope glycoprotein without other structural or regulatory proteins of HIV) has been described (16) and was used for immunizing the mice to induce envelope specific CTL.

Peptide Synthesis and Purification. Most of the peptides were prepared by the multiple simultaneous peptide method of solid-phase peptide synthesis, in polypropylene mesh "tea-bags" as described (17). Peptides were desalted by reverse-phase chromatography on C18 Sep-Pak columns (Waters Associates, Milford, MA), and analyzed by HPLC. Some peptides were prepared by an automated peptide synthesizer (model 430A; Applied Biosystems, Inc., Foster City, CA) and purified by HPLC. All of the peptides contained >90% of the desired product.

Exon-shuffled Transfectants. Mouse L cell clones stably transfected with H-2D^d and H-2L^d genes (T4.8.3 and T1.1.1, respectively) have been previously described (18). Transfectants T37.2.1 (expressing the D^d, D^d, and L^d derived sequences for the α1, α2, and α3 domains, respectively) (19), T37.1.3 ($L^dL^dD^d$) (20), T9.10.3 ($D^dL^dL^d$) (20), and DMT34.5 ($L^dD^dD^d$) (21) have been described in detail elsewhere. DMT26.5S1 expresses a cell surface protein lacking the α1 domain, but containing all other H-2D^d sequences (McCluskey, J., and D. H. Margulies, unpublished observations). All transfectant cell lines were examined by FACS analysis with an appropriate panel of anti-H-2D^d and anti-H-2L^d mAbs to confirm their expressed phenotype before the performance of the functional studies reported here.

CTL Generation. BALB/c mice (Jackson Laboratories, Bar Harbor, ME) were immunized intravenously with 10^7 PFU of recombinant vaccinia virus. 3–14 wk later, immune spleen cells (5×10^6/ml in 24-well culture plates in complete T cell medium (CTM)[1] a (1:1 mixture of RPMI 1640 and EHAA medium containing 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 5×10^{-5} M 2-ME) were restimulated for 6 d in vitro with either 2.5×10^6/ml of recombinant vaccinia virus-infected syngeneic spleen cells (1 h, 37°C, multiplicity of infection [MOI] of 10:1) or 2×10^5/ml of gp160 gene-transfected, MHC-identical fibroblasts (12). A long-term CTL line was also generated by repetitive stimulation of immune cells with mitomycin C-treated (100 μg/ml for 30 min at 37°C), gp160

gene-transfected H-2^d fibroblasts and 10% rat Con A supernatant-containing medium (rat T cell Monoclone; Collaborative Research, Inc., Bedford, MA).

Cloning of CTL. CTL clones were derived from the bulk lines through limiting dilutions onto mitomycin C-treated HIV-1-IIIB gp160 envelope gene transfected BALB/c 3T3 fibroblasts as stimulator cells in the presence of 10% rat Con A supernatant in U-bottomed 96-well plates at 0.3 cells/well and four clones (RT-1, RT-2, RT-3, RT-4) were obtained. Although the stimulator cells expressed whole gp160 envelope protein, all the clones were specific for the same 315–329 region (Takahashi, H., unpublished observations).

CTL Assay. Cytolytic activity of in vitro secondary CTL, CTL lines, or CTL clones was measured as previously described (12) using a 6-h assay with various ^{51}Cr-labeled targets, as indicated in the legends. For testing the peptide specificity of CTL, effectors and ^{51}Cr-labeled targets were mixed with various concentrations of peptide at the beginning of the assay (22) or effectors were cocultured with peptide-pulsed targets. The percent specific ^{51}Cr release was calculated as 100 × [(experimental release − spontaneous release)/(maximum release − spontaneous release)]. Maximum release was determined from supernatants of cells that were lysed by addition of 5% Triton-X 100. Spontaneous release was determined from target cells incubated without added effector cells.

Inhibitory Effect of Several Peptides on the Presentation of 18IIIB at the Target Cell Level. Class I D^d molecule-expressing fibroblasts were preincubated with competitor peptides at 20 μM overnight in the presence of 100μCi/ml of ^{51}Cr, then without removal of the competitor and ^{51}Cr, incubated with 1μM of the epitope peptide 18IIIB (315–329) at 37°C for 1 h. After the incubation, target cells were washed three or four times before use in the cytotoxic assay.

Results

Presentation of the Epitope Depends on Both α1 and α2 Domains of the D^d Molecule. In previous studies, we have found that residues 315–329 of the HIV-1 IIIB envelope protein constitute an immunodominant site for recognition by $CD8^+$ CTL of H-2^d mice, and that this site is seen only with the class I molecule D^d (12). Because L^d, which is homologous to D^d, does not present this peptide (12), we could use recombinant D^d and L^d molecules to determine which domains of the D^d molecule were specifically required for presentation of the peptide. The series of transfectants made in H-2^k L cell fibroblasts and expressing recombinant gene products is shown in Table I. We used peptide-pulsed ^{51}Cr-labeled cells as targets of cytolysis by H-2D^d-restricted peptide 18IIIB-specific cytotoxic T cell lines and clones, to avoid self-presentation of peptide by the H-2^d T cells. Only T37.2.1 ($\alpha1\alpha2$ of D^d) was sensitized with the peptide to the same extent as the positive control T4.8.3 L cell transfectant expressing a class I molecule with all D^d domains (Table I). Because DMT26.5S1 and DMT34.5 were not sensitized with the peptide, α1 deletion or α1 substitution with the corresponding L^d domain abrogated the recognition of the peptide. Conversely T9.10.3, with only α1 from D^d, was not sensitized by the peptide. This result showed that the D^d α1 domain alone was not sufficient. Therefore, we conclude that both specific α1 and α2 domains of D^d are necessary and together sufficient in the context of a complete mouse class I molecule to present the peptide and allow recognition by specific CTL.

The Effects of Single Amino Acid Substitutions on CTL Recognition. The CTL specific for the immunodominant site of the HIV-1-IIIB gp160 envelope protein represented by a 15-residue synthetic peptide (18IIIB) (315–329: RIQRGPGRAFVTIGK) did not crossreactively kill H-2^d targets infected with recombinant vaccinia virus expressing the envelope gene from the natural HIV-1-RF variant (15) or targets pulsed with a peptide corresponding to the homologous site in the HIV-1-RF gp160 enve-

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TABLE I

Both the α1 and α2 Domains of the D^d Molecule Are Necessary and Sufficient for Presentation and Recognition of the 18IIIB Determinant

L cell transfectant targets*	Class I D^d molecules				Percent specific lysis‡	
	α1	α2	α3	TM§	LINE‖	RT-1^d‖
T4.8.3	D^d	D^d	D^d	D^d	25.9	25.2
T1.1.1	L^d	L^d	L^d	L^d	1.9	1.9
DMT26.5S1	–	D^d	D^d	D^d	3.8	5.2
T37.2.1	D^d	D^d	L^d	L^d	27.1	34.0
T37.1.3	L^d	L^d	D^d	D^d	-4.9	-2.9
T9.10.3	D^d	L^d	L^d	L^d	-6.9	-7.5
DMT34.5	L^d	D^d	D^d	D^d	3.5	1.5

* Target cells were pulsed overnight with 1 μM of peptide 18IIIB and washed four times to remove free peptide.
‡ Target ratio = 5/1.
§ TM: transmembrane portion of the D^d molecule.
‖ Effectors are CTL-LINE and clone RT-1 (see Materials and Methods).

lope protein (18RF) (315–329: --HIGPGRVIYATGQ). Therefore, by examining the role of each residue at which these variants differ, we could both identify the residues involved in interaction with the MHC molecule or TCR and also examine the structural basis for the effect of viral variation on T cell reactivity. We synthesized a series of peptides with single amino acid substitutions at each position in which 18IIIB and 18RF differ or with an alanine substitution at positions conserved in the two HIV isolates (Table II) to determine which substitutions affect interaction with the TCRs of the CTL-LINE and clones (RT-1, RT-2, RT-3) that we have developed, or

TABLE II

Substituted Peptides Used for Mapping Peptide Binding/Recognition

Sequence position	315														329
No.18 IIIB	R	I	Q	R	G	P	G	R	A	F	V	T	I	G	K
No.18 RF	*	*	T	K	G	P	G	R	V	I	Y	A	T	G	Q
No.18-1	*‡	I	Q	R	G	P	G	R	A	F	V	T	I	G	K
No.18-2	R	*‡	Q	R	G	P	G	R	A	F	V	T	I	G	K
No.18-3	R	I	T	R	G	P	G	R	A	F	V	T	I	G	K
No.18-4	R	I	Q	K	G	P	G	R	A	F	V	T	I	G	K
No.18-5	R	I	Q	R	A	P	G	R	A	F	V	T	I	G	K
No.18-6	R	I	Q	R	G	A	G	R	A	F	V	T	I	G	K
No.18-7	R	I	Q	R	G	P	A	R	A	F	V	T	I	G	K
No.18-8	R	I	Q	R	G	P	G	A	A	F	V	T	I	G	K
No.18-9	R	I	Q	R	G	P	G	R	V	F	V	T	I	G	K
No.18-10	R	I	Q	R	G	P	G	R	A	I	V	T	I	G	K
No.18-11	R	I	Q	R	G	P	G	R	A	F	Y	T	I	G	K
No.18-12	R	I	Q	R	G	P	G	R	A	F	V	A	I	G	K
No.18-13	R	I	Q	R	G	P	G	R	A	F	V	T	T	G	K
No.18-14	R	I	Q	R	G	P	G	R	A	F	V	T	I	A	K
No.18-15	R	I	Q	R	G	P	G	R	A	F	V	T	I	G	Q

* Indicates a deletion.
‡ The underlined amino acids are substitutions in the 18IIIB sequence.

with the D^d molecule. The results are summarized in Figs. 1 and 2. Although there is some shift in the dose-response curves for most of the substituted peptides (18-3 to 18-13) compared with the wild-type peptide 18IIIB, the data clearly demonstrated that substitutions only at positions 322(R) (18-8), 324(F) (18-10), and 325(V) (18-11) markedly affect CTL activity. In comparing the activities of clone RT-1 and RT-3, substitutions of 322(R) with Ala and of 325(V) with Tyr were each less detrimental for clone RT-3 than clone RT-1, but substitution of 324(F) with Ile abrogated the CTL response completely for both clones. The variable effect of substitutions at positions 322 and 325 for different clones (Fig. 2) was consistent with the partial loss of activity for the CTL-LINE (Fig. 1), whereas the uniform effect of substitution at position 324 for the clones was consistent with complete loss of activity for the line. These effects could be due to interference with peptide binding to class I MHC molecules, or to interference with TCR recognition. To distinguish these mechanisms, we measured competition for class I MHC molecule presentation.

Competition Analysis of the Substituted Peptides with Diminished Activity in the CTL Response. Substitution of 322(R) with Ala and 324(F) with Ile and 325(V) with Tyr decreased or eliminated the ability of the peptide to sensitize target cells. To investigate whether the decreased activity is due to diminished D^d binding or to changes in the interaction with the TCR, we pulsed the D^d-expressing targets with high concentrations of the substituted peptides together with ^{51}Cr overnight and then added

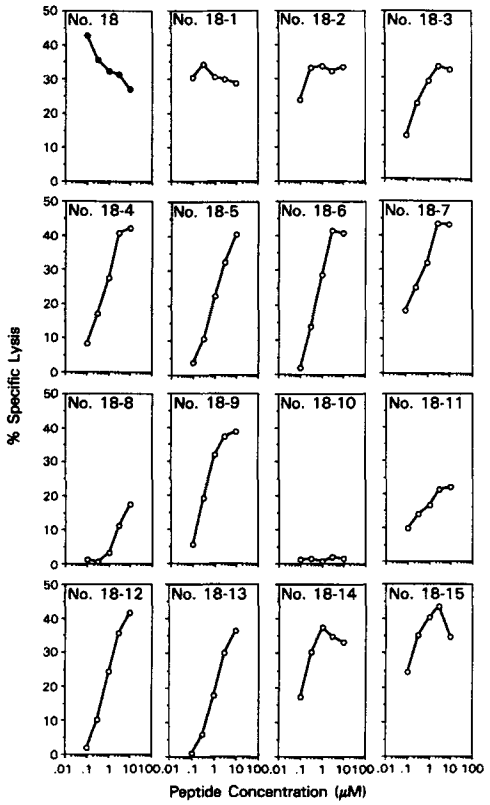

FIGURE 1. The effects of single amino acid substitutions on recognition of variant epitopes by a IIIB-specific CTL-LINE. Peptide 18IIIB-specific CTL-LINE was added to ^{51}Cr-labeled BALB/c 3T3 fibroblast target cells in the presence of indicated concentrations of substituted peptides as shown in Table II. E/T ratio was 5:1 in each case.

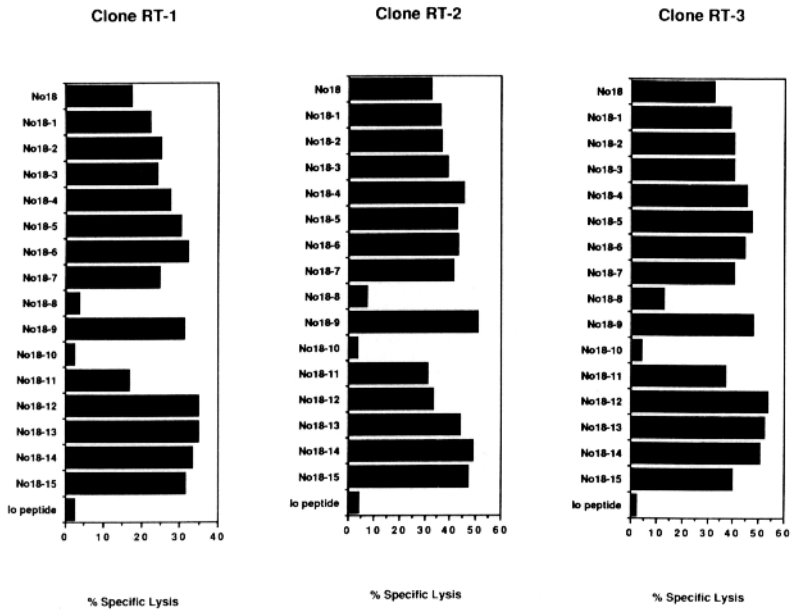

FIGURE 2. The effects of single amino acid substitutions on peptide sensitization of targets for recognition by individual CTL clones. Clones RT-1, RT-2, and RT-3 specific for peptide 18IIIB were tested for recognition of BALB/c 3T3 fibroblast targets in the presence 10 μM of the substituted peptides as shown in Table II. E/T ratio was 5:1 in each case.

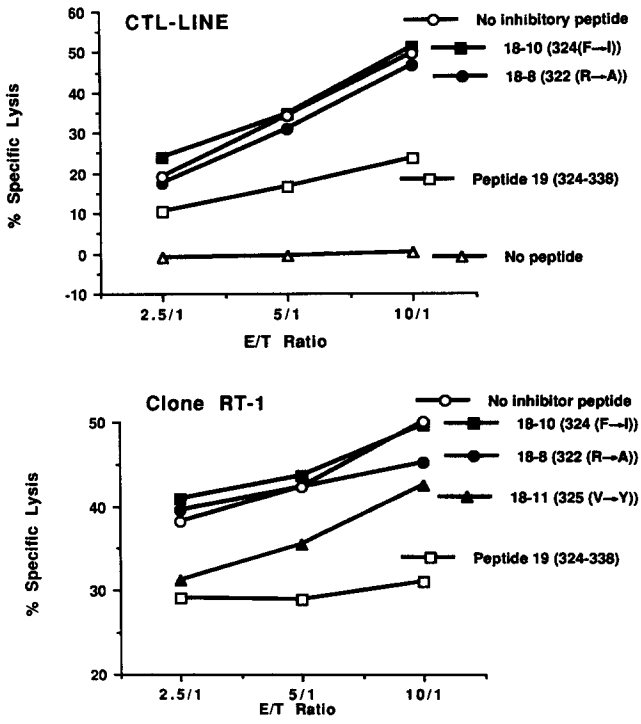

FIGURE 3. Peptides substituted at 322 and 324 fail to compete with wild-type peptide 18IIIB for presentation to CTL. Competition method was as follows: BALB/c 3T3 fibroblasts (H-2^d) were preincubated with competitor peptides at 20 μM overnight in the presence of 100 μCi/ml of ^{51}Cr, then without removal of the competitor and ^{51}Cr, incubated with 1 μM of the epitope peptide 18IIIB (315-329) at 37°C for 1 h. After the incubation, target cells were washed three to four times before use in the 6-h cytotoxic assay. Although No. 18-8 (322 (R → A)) (●) and No. 18-10 (324 (F → I)) (■) did not inhibit the presentation of 18IIIB, No. 18-11 (325 (V → Y)) (▲) did partially inhibit the presentation. Peptide 19 (324-338) □) was used as a positive inhibitor control. Standard errors of the means of triplicate cultures were always <5% of the mean.

1 μM 18IIIB for 60 min. After the incubation, cells were harvested and washed carefully four times to remove free peptides before being used as targets. As Fig. 3 shows, we could not detect any significant inhibitory activity of peptides substituted at either 322 or 324 for the CTL-LINE or clone. As a control, we show that the overlapping peptide 19, residues 324–338, can inhibit under these conditions. As failure to compete during pulsing suggests failure to bind the MHC molecule, these results suggest that positions 322(R) and 324(F) may both be involved in determining the extent of peptide binding to the D^d class I MHC molecule. In contrast, the peptide with substitution of 325(V), which strongly affected response of only one of the CTL clones, did partially inhibit presentation of wild-type 18IIIB to this clone (Fig. 3, *bottom panel*). Since at high concentrations this peptide sensitizes targets for all of the CTL clones, it is not possible to achieve complete inhibition. Since ability to compete during pulsing of targets with peptide, in the absence of T cells, generally indicates competition for MHC molecule binding and therefore the ability to bind, these data suggest that 325(V) affects interaction with the TCR. Also, successful competition by overlapping peptide 19 (324–338) indicates that this peptide binds to D^d even though it does not sensitize targets. The simplest explanation is that peptide 19 shares enough of the D^d binding structure of peptide 18IIIB to bind. It is formally possible that this peptide has a distinct D^d binding site in residues 330–338, which are not seen because of a hole in the T cell repertoire. However, the observed scarcity of peptides presented by D^d in this molecule (12) and the rarity of holes in the T cell repertoire (23) mean that two relatively unlikely events would have to coincide for the latter interpretation to hold.

Discussion

Using a series of overlapping peptides of the HIV-1-IIIB gp160 envelope glycoprotein, we have previously identified an immunodominant site for CTL recognition (12), based on the methods of Townsend et al. who showed that antigens recognized by CTL can be mimicked by synthetic peptides (22). The immunodominant epitope recognized by H-2^d mice consists of a 15-residue sequence (18IIIB: RIQRGPGRAFVTIGK). By use of two H-2^k L cell fibroblast transfectants, we demonstrated that recognition of the epitope 18IIIB is restricted by the class I D^d molecule, not the L^d molecule. We took advantage of this well-defined system to determine structural requirements of CTL activity for both the class I molecules and the antigenic peptide, and to explore the structural basis for the effect of viral variation on T cell reactivity.

It has already been reported that allospecific and influenza virus–specific CTL are restricted to the α1 and/or α2 domain of class I molecules (13, 24). It has also been reported that the frequency of murine CTL precursors (CTLp) that recognize the human histocompatibility antigen HLA-A2 and HLA-B7 is controlled by structures within α1 and α2 domains of the class I molecules (25). Although the three-dimensional structure of the class I molecule (26, 27) makes it likely that both α1 and α2 domains may play some role in peptide binding, previous experiments indicate that the α1 domain of D^d is sufficient for allorecognition by anti-H-$2D^d$ CTL when associated with the α2 and α3 domains of L^d (21). To determine whether the response to the immunodominant epitope of the HIV-1-IIIB envelope protein also depends on both the α1 and α2 domains of the D^d class I molecules, we used sev-

eral L cell (H-2^k) transfectants with different exon shuffles between D^d and L^d (Table I). The results revealed that the 18IIIB peptide requires both the α1 and α2 domains of the D^d molecule for effective presentation. These two domains of D^d alone are sufficient, in the context of a complete murine class I molecule, for presentation of the immunodominant site. Furthermore, neither the α1 nor the α2 domain of D^d, when the other derives from L^d, is alone sufficient for presentation of the peptide. We conclude that both the α1 and the α2 domains of the D^d molecule are necessary and together sufficient in an intact class I MHC molecule for presentation of the epitope. Previous studies of class I molecules with exon shuffles (19–21, 24, 25) have largely dealt with allorecognition, and in particular, have not dealt with presentation of a defined peptide. A recent study by McMichael et al. (28) using site-directed mutants of the human class I molecule HLA-A2.1 also suggests that residues of both the α1 and α2 domains play a role in binding of a peptide from the influenza matrix protein. Very recently, Martinon et al. (29), using H-$2K^d$/D^d recombinant molecules, have demonstrated the importance of residues 152, 155, and 156 in the α2 domain of K^d in the presentation of an HLA peptide. However, without reciprocal recombinants, this study was unable to address the role of the α1 domain of K^d.

A comparison of the residues that differ between H-$2D^d$ and H-$2L^d$ in the α1 and α2 domains (30) and consideration of their likely structural relationship based upon the three-dimensional structure of the homologous human HLA-A2.1 molecule (26, 27) and of a model of the murine H-$2K^b$ molecule (31) offer some understanding of the residues critical for binding of the HIV peptide 18IIIB to D^d or to the H-$2D^d$-restricted CTL. Of the 18 residues that differ between H-$2D^d$ and H-$2L^d$ in the α1 domain, and the 8 residues that differ in the α2 domain, only 10 are likely to be either facing the antigen binding cleft or directly accessible to the TCR and are nonconservative. Thus, positions 9, 31, 63, 65, 66, 73, 77, 114, 155, and 156 are likely to play an important role in either the interaction of peptide 18 with the H-$2D^d$ molecule, or of the peptide/MHC complex with the TCR. Further experiments using an in vitro mutagenesis approach should clarify the role of each of these positions.

Because the IIIB CTL determinant lies in a region of gp160 that is highly variable among the different HIV-1 isolates (32), we could use naturally occurring substitutions in the gp160 sequence to define the roles of individual peptide residues in immune recognition. The CTL line specific for peptide 18IIIB from the HIV-1-IIIB isolate did not crossreactively kill targets infected with recombinant vaccinia virus expressing the envelope gene from the RF isolate or targets pulsed with the homologous peptide of the RF gp160 envelope protein (18RF). As shown in Table II, a series of peptides that differ by each of these substitutions individually was made to determine which substitutions were responsible for the lack of response of the anti-HIV-1-IIIB CTL to the HIV-1-RF peptide. The results clearly showed that substitutions of 322(R) by Ala and 324(F) by Ile markedly reduced the CTL recognition of the peptide, and the latter substitution appeared to be more critical (Figs. 1 and 2). Moreover, neither of these two substituted peptides inhibited the binding of the original peptide 18IIIB (Fig. 3). Therefore, we may conclude that, among the 15-residues of the peptide, 322(R) and 324(F) may be critical in controlling binding to the D^d class I molecule (agretopic amino acids). The data also suggest that residue 325(V) may be involved in TCR interaction. Furthermore, in spite of not containing the agretopic residue 322(R), the peptide 324–338 seems to be able to bind to the D^d

molecule (Fig. 3). Taking everything together, the critical residues for binding to D^d and also to the TCR appear to be between residues 324 and 329. While 322(R) is not contained in this region, it appears to also contribute to the binding to D^d, or at least its substitution with Ala interferes either directly or via a conformational effect. Interestingly, our results are compatible with the recent results of Gotch et al. (10) on presentation of a peptide of influenza matrix protein by HLA-A2. These investigators found that the most effective substitutions for abrogating the ability to sensitize the targets were substitutions at an agretopic residue (residue 61), i.e., one that was found to be part of the site binding to the class I MHC molecule. However, the observation that only one or two residues are critical for class I presentation is somewhat surprising in view of the finding for class II-restricted presentation that in some experiments has been shown to be affected by substitutions at each of several positions (33, 34), and in view of fraction of the peptide structure that could be enclosed within the MHC groove (26, 27).

The peptide 18IIIB has a sequence with the right periodicity of hydrophobicity to form an amphipathic α-helix. Although the GPG sequence in the immunodominant epitope will probably induce a bend in the predicted helix, the region starting from GPG through the COOH terminus can form three amphipathic α-helical turns. The data suggest that this is the critical region for class I MHC molecule binding and, therefore, for CTL recognition, consistent with the helical amphipathicity hypothesis (35–37).

Because this same HIV peptide is also recognized by human CTL (14), these results may have clinical implications for HIV infection. Comparing the sequence of this immunodominant CTL determinant in the HIV-1-IIIB sequence with the corresponding region in the sequence of the HIV-1-RF isolate, it is of interest that changes appear to take place in both the residues that are required for class I MHC binding (agretopic residues) and residues that determine T cell specificity or TCR interaction (epitopic residues). Although a mutant virus may not have a selective advantage against CTL in the cell in which it arises, in the midst of other wild-type virions, if it buds before that cell is lysed and infects another cell at a multiplicity of one, it would have an advantage in escaping CTL. Changes in epitopic residues might affect susceptibility of the virus to immune defense mechanisms if the T cell repertoire varies among individuals, although the repertoires are thought to be broad enough that this may be relatively rare. Nevertheless, the rarity of class I MHC-restricted epitopes in several proteins (12, 38, 39) suggests that perhaps the repertoire of determinants recognized in association with class I MHC molecules is less broad than expected. Changes in agretopic residues would be expected to have a more profound effect in that they would determine which individuals, of different HLA types, could raise any CTL response at all against this immunodominant epitope in association with their class I molecules. Furthermore, it has been shown that the HIV virus can mutate within the course of disease in a single individual (40, 41). If mutations occur only in epitopic residues, existing CTL may be made ineffectual, but new CTL could be elicited that see the new mutant epitope with the same MHC molecule. On the other hand, if mutations occur in agretopic residues, it is possible that the mutant determinant may not be able to be presented by any of the class I MHC molecules of that individual. Thus, if only a single CTL determinant is available on that protein of the virus, as appears to be the case here for

the HIV envelope as seen by H-2^d mice, the individual would become immunologically blind to that protein of the mutant virus with respect to class I-restricted effector cells. We might speculate, then, that if class I-restricted CTL are an important host mechanism keeping HIV in check during the usually long latency period of the infection (42), such a mutational event in which the mutant virus becomes at least partially immunologically invisible to the host may allow the virus to escape immune control, replicate rapidly, and cause AIDS. Of course, CTL specific for other proteins of the virus might prevent this, but if only a few proteins are critical targets for CTL and if each of these has only one or two immunodominant epitopes, then mutation of just a few of these epitopes may be enough to tip the balance and allow virus to escape control in that individual. For these reasons, it would be valuable to compare sequences of such epitopes in serial isolates of virus from the same patient as the disease progresses.

Summary

In H-2^d mice, the immunodominant determinant of the HIV-1-IIIB gp160 envelope glycoprotein recognized by $CD8^+$ CTL is represented by a 15-residue synthetic peptide (315-329: RIQRGPGRAFVTIGK). This peptide is seen in association with the D^d class I MHC molecule expressed on H-2^k L cell fibroblast targets. We explored the structural requirements for CTL recognition of this peptide at the levels of both the peptide molecule and the class I MHC molecule. Using several transfectants expressing recombinant D^d/L^d molecules, we found that presentation of this epitope required both the $\alpha 1$ and $\alpha 2$ domains of the D^d molecule, in contrast to certain instances of allorecognition for which $\alpha 1$ of D^d was sufficient in association with $\alpha 2$ of L^d.

Because this peptide derives from a hypervariable segment of the HIV envelope, substituted peptides could be used to define not only the structures affecting interaction of peptide with class I MHC molecule and with the TCR, but also the structural basis for the effect of naturally occurring viral variation on CTL recognition. The CTL-LINE specific for this HIV-1-IIIB-derived sequence could not recognize the HIV-1-RF variant-derived sequence from exactly the same site (315-329: --HIGPGRVIYATGQ). Peptides with single amino acid substitutions from the HIV-1-IIIB sequence toward the HIV-1-RF sequence were made to test the effect of each residue on the ability to sensitize targets. The results clearly showed that only three residues significantly affected recognition, and only one, 324(F), was obligatory. Moreover, both 322(R) and 324(F) substituted peptides failed to inhibit the binding of the wild type peptide to the MHC molecule. Therefore, the amino-acids 322(R) and 324(F) seem to be involved in regulating peptide interaction with the D^d class I MHC molecule. In contrast, 325(V) appeared to affect interaction with the TCR. We suggest that sequence variations among known HIV-1 isolates that affect peptide binding to MHC such as those described here, if occurring during the course of infection of an individual, could result in failure of the MHC molecules of that individual to present the peptide. If the number of dominant HIV CTL epitopes is indeed very limited, such a blind spot could allow the virus to escape immune control, proliferate rapidly, and cause AIDS.

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