Structural Basis for Degenerate Recognition of Natural HIV Peptide Variants by Cytotoxic Lymphocytes* GHz

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It is well established that even small changes in amino acid side chains of antigenic peptide bound to major histocompatibility complex (MHC) protein may completely abrogate recognition of the peptide-MHC (pMHC) complex by the T cell receptor (TCR). Often, however, several nonconservative substitutions in the peptide antigen are accommodated and do not impair its recognition by TCR. For example, a preponderance of natural sequence variants of the human immunodeficiency virus p17 Gag-derived peptide SLYNTVATL (SL9) are recognized by cytotoxic T lymphocytes, which implies that interactions with SL9 variants are degenerate both with respect to the class I MHC molecule and with respect to TCR. Here we study the molecular basis for this degenerate recognition of SL9 variants. We show that several SL9 variants bind comparably well to soluble HLA-A2 and to a particular soluble TCR and that these variants are active in the cognate cytotoxicity assay. Natural SL9 variation is restricted by its context in the HIV p17 matrix protein. High resolution crystal structures of seven selected SL9 variants bound to HLA-A2 all have remarkably similar peptide conformations and side-chain dispositions outside sites of substitution. This preservation of the peptide conformation despite epitope variations suggests a mechanism for the observed degeneracy in pMHC recognition by TCR and may contribute to the persistence of SL9-mediated immune responses in chronically infected individuals.

Antigen-specific receptor on T lymphocytes (TCR) recognizes a composite ligand consisting of antigenic peptide and MHC moiety (pMHC). Originally thought to be completely selective for a given pMHC complex, each TCR is now known to recognize a variety of natural pMHC ligands.

For example, cytotoxic T lymphocytes (CTL) from the majority of HIV-infected patients recognize a specific Gag-derived peptide in the context of HLA-A2 class I MHC protein (1, 2). The presented peptide includes residues 77–85 of p17, the matrix protein encoded by the gag gene. In canonical B-clade viruses such as HxBc2, this is a nonamer with the sequenced M-group HIV-1 isolates (HIV sequence data base, hiv-web.lanl.gov), and many SL9-specific CTL clones from infected individuals recognize the majority of these variants with very few exceptions (3), albeit with different sensitivity (2). This indicates that most amino acid substitutions in the SL9 epitope, which result from virus mutations, do not abolish the recognition of the pMHC complexes by the TCR. To investigate the basis of degenerate recognition of the HIV Gag epitope by CTL, we analyzed the interaction between soluble TCR from the SL9-specific CTL clone D3 and soluble HIV-A2 protein loaded with SL9 variants as an oligomeric complex and solved high resolution crystal structures of seven SL9 peptide variants bound to HLA-A2. The analysis revealed remarkably similar tertiary structures for all MHC-bound peptides. Importantly, this common conformation was distinct from that of other HIV-A2-bound peptides. This uniquely conserved structure of the peptides and the nature of amino acid residues at critical peptide positions regardless of other natural substitutions suggest a mechanism for the observed degeneracy in recognition.

EXPERIMENTAL PROCEDURES

Proteins and Enzyme-linked Immunosorbent Assay—Expression and purification of soluble D3 TCR and HLA-A2-containing site for biotinylation were performed as described (4, 5).
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The HLA-A2 was biotinylated using biotin transferase (avidity) according to the manufacturer’s protocol, and the HLA-A2-peptide/tetramer was produced as described (6, 7) using a streptavidin-peroxidase conjugate (Sigma). Binding of various peptide-HLA-A2 complexes to soluble D3 TCR was evaluated as described previously (4, 5). Briefly, 96-well plates were coated with soluble TCR at 12 μg/ml at 4 °C overnight and blocked with 1% bovine serum albumin/PBS for 2 h at room temperature. Peptide-HLA-A2 tetramers were diluted in 1% bovine serum albumin/PBS and added to the plates in triplicate at various concentrations. The excess of the peptide (about 10^{-5} M) was kept in solution to ensure saturation of HLA-A2 molecules. After 2 h of incubation at room temperature, the plates were washed three times with iced PBS/Tween and cold PBS, and the assay was developed with o-phenylenediamine; the absorbance was measured at 490 nm. For crystallization purposes, the biotin-receptive site was deleted, and the HLA-A2 was expressed, purified, and loaded with peptides as above.

Cells and Cytolytic Assay—The CD8^+ HIV Gag-specific D3 CTL and the HLA-A2^+ Epstein-Barr virus-transformed B cell line JY were maintained in culture as described (2). ^{51}Cr-labeled JY cells (5 × 10^3 cell/well) were incubated in triplicate with designated dilutions of peptides in complete medium in the presence of D3 CTL at a CTL/target cell ratio of 5:1 in 96-well round bottom plates. Control wells containing either ^{51}Cr-labeled JY cells and CTL without peptide or with irrelevant peptide or the labeled cells in the presence of detergent or medium alone were prepared and carried out in quadruplicate. After 4 h at 37 °C under 5% CO_2, 100-μl aliquots from each supernatant were counted on a γ-counter (Wallac), and percent specific lysis was determined as described previously (^{51}Cr release into supernatant - spontaneous release)/(total release in detergent - spontaneous release) × 100 (8).

Crystallization—Orthorhombic crystals of the HLA-A2-SL9 complex were grown by the hanging-drop method at 20 °C by suspending 2 μl of 10 mg/ml protein mixed with 2 μl of 1.6 M (NH_4)_2SO_4, 10 mM CoCl_2, and 100 mM MES, pH 6.5. The monoclinic crystals were prepared by microseeding. The seeding solutions were prepared by crushing the orthorhombic crystals with a glass rod and serially diluting this mixture with a buffer containing 15% polyethylene glycol 6000 and 25 mM MES, pH 6.5. The monoclinic crystals were grown by the hanging-drop method at 20 °C by suspending 2 μl of 10 mg/ml protein mixed with an equal volume of the seeding solution. The drops were equilibrated over a reservoir buffer containing 15% polyethylene glycol 6000 and 25 mM MES, pH 6.5. Crystals reached the maximum size (SL9-HLA-A2, 300 × 200 × 20 μm) within 1–2 days.

Data Collection and Processing—The orthorhombic crystals were transferred into cryoprotectant buffer containing 1.6 M (NH_4)_2SO_4, 10 mM CoCl_2, 100 mM MES, pH 6.5, 20% glycerol, and 10% ethylene glycol, pH 6.5. Monoclinic crystals were transferred into cryoprotectant buffer containing 20% polyethylene glycol 6000, 25 mM MES, 0.02% NaNO_3, and 20% glycerol, pH 6.5. All crystals were frozen at 100 K in cold nitrogen. Diffraction data for all complexes were collected on beamline X4A at the National Synchrotron Light Source (Brookhaven, NY) using a Quantum 4 charge-coupled device (Area Detector Systems Corp., Poway, CA). Diffraction data were processed with DENZO and SCALEPACK (9). Data collection statistics, including lattice parameters, are shown in supplemental Table 1S.

Structure Determination and Refinement—The structure of SL9-HLA-A2 complex in the orthorhombic space group was determined by molecular replacement in CNS (10) using free HLA-A2/Tax as the search model (11). The structure of the monoclinic crystal was solved by molecular replacement using the refined orthorhombic HLA-A2 structure. Packing in the orthorhombic lattice is like that found by Bjorkman et al. (12, 13), but the monoclinic packing is unique despite cell dimension similarities (14). All other monoclinic complexes were determined by rigid body refinement, using the refined monoclinic HLA-A2 structure as a starting model. In all cases, peptides were added to empty HLA-A2 models by fitting into simulated-annealing omit maps, and simulated annealing was again used at the start of refinement to reduce model bias. Building was performed with the modeling program O (15). The final refinement statistics for all structures are presented in supplemental Table 1S. Atomic coordinates have been deposited in the Protein Data Bank, and Protein Data Bank codes...
TABLE 1
Recognition of natural and synthetic SL9 variants by SL9-specific D3 TCR

| Peptide   | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | SD_{50} \text{M} | IC_{50} \text{nM} | DR_{50} \text{nM} |
|-----------|----|----|----|----|----|----|----|----|----|-----------------|-----------------|-----------------|
| Natural variants |
| SL9       | S  | L  | Y  | N  | T  | V  | A  | T  | L  | 8 \text{E}11 | 50 \text{nM} | 5-7             |
| SL9-6I    | -  | -  | I  | -  | -  | V  | -  | -  | -  | 1 \text{E}8   | 620 \text{nM} | 30              |
| SL9-6I/8V | -  | -  | I  | -  | V  | -  | -  | -  | -  | 2 \text{E}10   | 39 \text{nM} | 5-7             |
| SL9-8V    | -  | -  | -  | -  | -  | V  | -  | -  | -  | 2 \text{E}10   | 128 \text{nM} | 5-7             |
| SL9-9I    | -  | F  | -  | -  | -  | I  | -  | -  | -  | 3 \text{E}10   | 1339 \text{nM} | 5-7             |
| SL9-9I/6I | -  | F  | I  | -  | -  | V  | -  | -  | -  | 4 \text{E}10   | 94 \text{nM} | 5-7             |
| SL9-9I/6V | -  | F  | I  | -  | -  | V  | -  | -  | -  | 3 \text{E}10   | 1014 \text{nM} | 5-7             |
| SL9-9I/8V | -  | F  | I  | -  | -  | V  | -  | -  | -  | 1 \text{E}10   | 54 \text{nM} | 10              |
| SL9-9S    | -  | A  | -  | -  | -  | -  | -  | -  | -  | >10^8          | 29 \text{nM} | >1000           |
| SL9-9S/6I | -  | A  | I  | -  | -  | -  | -  | -  | -  | 7 \text{E}10   | 55 \text{nM} | 12              |
| SL9-9S/6V | -  | L  | -  | -  | -  | V  | -  | -  | -  | >10^8          | 36 \text{nM} | >1000           |
| SL9-9S/8V | -  | L  | -  | -  | -  | V  | -  | -  | -  | >10^8          | 27 \text{nM} | >1000           |
| Synthetic variants |
| SL9-9Q    | -  | Q  | -  | -  | -  | -  | -  | -  | -  | 2 \text{E}7    | 28 \text{nM} | 70              |
| SL9-4L    | -  | L  | -  | -  | -  | -  | -  | -  | -  | 4 \text{E}10   | 36 \text{nM} | 15              |
| SL9-1A    | A  | -  | -  | -  | -  | -  | -  | -  | -  | 8 \text{E}10   | 67 \text{nM} | 5-7             |
| SL9-3A    | A  | -  | -  | -  | -  | -  | -  | -  | -  | >10^8          | 42 \text{nM} | >1000           |
| SL9-3A    | A  | -  | -  | -  | -  | -  | -  | -  | -  | >10^8          | n.d.          | >1000           |
| SL9-5V    | -  | V  | -  | -  | -  | -  | -  | -  | -  | >10^8          | 23 \text{nM} | >1000           |
| SL9-6A    | A  | -  | -  | -  | -  | -  | -  | -  | -  | 1 \text{E}7    | 91 \text{nM} | 50              |
| SL9-8A    | -  | A  | -  | -  | -  | -  | -  | -  | -  | 3 \text{E}8    | 54 \text{nM} | 40              |
| SL9-9A/6A/8A | A | -  | A  | -  | -  | -  | -  | -  | -  | >10^8          | 160 \text{nM} | >1000           |

1) Peptide concentration in the extracellular medium that is required to achieve half-maximal lysis of JY cells by D3 CTL.
2) Peptide concentration that is required to block binding of radiolabeled reference peptide to soluble HLA-A2 by 50% or to inhibit by 50% the response of HLA-A2-restricted CTL against irrelevant peptide.
3) Concentration of HLA-A2/tetramer is defined by the concentration of monomeric pMHC complexes in the preparation of the tetramer.
4) Values were reported previously (2).
5) Values were estimated in the peptide competition assay (8) using SL9 as a reference peptide.
6) n.d. indicates not determined.

The majority of the SL9 natural variants were active in the cytotoxicity assay (Fig. 1, open symbols) (2). One exception was a single substitution of Ala for Thr in P5, which did not affect peptide binding to HLA-A2 (Table 1) but completely abolished CTL recognition (Fig. 1), indicating that P5 Thr plays a critical role in recognition of the SL9-HLA-A2 complex by D3 TCR. The natural SL9-3F variant was only slightly less active than wild type SL9 (Fig. 1 and Table 1).

We next examined synthetic variants with mutations at other positions. The variants with nonconservative substitutions of Ala for Tyr at P3 and Ala for Asn at P4 were not recognized by the TCR (Fig. 1 and Table 1). It is essential to note that no SL9 natural variants have substitutions at P4. An Asn in this position, which corresponds to position 80 in p17, appears to be conserved in all known viral isolates. Peptide SL9-1A was indistinguishable from SL9, whereas SL9-8A was somewhat less active; the peptide in which Val was replaced for Ala at P6 (SL9-6A) possessed very low activity (Table 1). A peptide having all three substitutions (SL9-1A/6A/8A) was not active at all (Table 1). These and other data (3) suggest that P6 Val contributes to peptide recognition by the TCR.

Interaction of Immobilized D3 TCR with HLA-A2/Tetramer Loaded with Natural SL9 Variants—The interactions between HLA-A2 loaded with SL9 variants and D3 TCR were evaluated in an enzyme-linked immunosorbent assay in which peroxidase-labeled pMHC tetramers are tested for binding to soluble D3 immobilized on the plastic surface (Fig. 2) (4, 5). The concentration of
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tetramer required for half-maximal response (DR50) was used to characterize the strength of each TCR-pMHC interaction; a lower DR50 value is associated with a stronger reaction (see Table 1). That tetramers containing peptides with substitution at P5 did not bind D3 TCR to a detectable extent establishes the essential role of Thr at P5 in the specificity of the D3-HLA-A2-SL9 reaction. Conservative P3 Tyr and P4 Asn also appeared to be critical for binding specificity; replacement of these amino acids with Ala completely eliminated binding. Substitutions in other peptide positions either had a small effect or did not influence binding at all (Table 1).

**Crystal Structure of HLA-A2 Associated with SL9 and -6 Peptide Variants**—To elucidate similarities and differences of the pMHC complexes recognized by SL9-specific TCR, we determined the x-ray crystal structures of the SL9-, SL9-3A-, SL9-3F/6I/8V-, SL9-4L-, SL9-5V-, SL9-6I-, and SL9-1A6A8A-HLA-A2 complexes to resolutions between 1.9 and 2.25 Å. The overall structures of the MHC molecules are essentially identical, as are the main chain conformations of SL9 and the six variants (see below). Crystallization of SL9-HLA-A2 in two different lattices results in the same peptide structure, indicating that the observed peptide conformation is not an artifact of crystallization but reflects the native conformation of the peptide bound to the MHC molecule.

All peptide structures showed distinct, unambiguous density, although peptide side chains at positions P4 and P5 were less clearly defined. A simulated annealing omit map for the SL9 peptide is shown in Fig. 3a. Fig. 3b shows the aromatic ring of the Tyr at P3 emerging from the binding groove and positioned along with P4 and P5 for recognition by the TCR. Replacement of the Tyr with Ala at P3 led to the formation of a cavity filled with two crystallographic water molecules in the SL9-3A complex (Fig. 3c). Similarly in the SL9-1A6A8A complex, replacement of the buried Val at P6 with Ala led to the formation of a different water-filled cavity in place of the Cγ2 methyl group (not shown). Because of the hydrophobic nature of Tyr and Val, replacement of the hydrophobic group by water may have an unfavorable effect on the TCR-pMHC interaction (4, 11), perhaps explaining the complete loss of detectable binding of SL9-A3-HLA-A2 and SL9-1A6A8A-HLA-A2 to D3 TCR (Table 1). In addition, the acquisition of these unique crystallographic water molecules in the SL9-3A- and SL9-1A6A8A-HLA-A2 structures presents exclusive hydrogen bonding partners to the backbone amide nitrogen and carbonyl oxygen at P4, respectively. As a consequence, the peptide plane acquires a modestly different, unique orientation. These examples illustrate the extent of structural changes of pMHC surface that may abate TCR recognition.

A top view of overlapping SL9 and six variants bound to HLA-A2 is depicted in Fig. 4a. The central region of the peptides is highly exposed to solvent similar to other peptide-MHC class I structures (18, 20), as evidenced by the arc of the peptide in positions P4, P5, and P6 seen in Fig. 4, b and c. The solvent accessibility of SL9 side chains as bound to HLA-A2 is shown in Fig. 4d. The exposed P4 and P5 side chains show some flexibility, reflected in weaker crystallographic electron density and higher than average atomic mobility factors (B-factors). Although the nonconservative substitution of P3 Tyr for Ala leads to a complete loss of the TCR-pMHC interaction, the backbones of this and other SL9 peptides are still indistinguishable.

**Comparison of the Structures of Peptides Bound to HLA-A2**—To evaluate the significance of peptide conformation in recognition of the SL9 group of peptides, we compared the structure of the SL9 peptide found here with the structures of other nonameric peptides bound to HLA-A2 (11, 12, 14, 18, 19, 21–30). As the measure of variation, we used the r.m.s.d. for main chain atoms at each peptide position. The variations between SL9 and other SL9 variants were compared with those between SL9 and other HLA-A2 peptides (Fig. 5).

By comparing SL9 to the natural and synthetic variants studied here, we found the structures of the peptides to be very similar, reflected in low r.m.s.d. for each residue (Fig. 5). The anchor residues at the N and C termini of the peptide are essentially identical (r.m.s.d. of ~0.2 Å), and although the central region is more deviant (~0.5 Å r.m.s.d.), reflecting the implicit flexibility of this segment, it is still quite similar. In contrast, comparison of the structure of SL9 to the structures of other HLA-A2-peptide complexes revealed greater deviations throughout the entire peptide span, particularly at the central region, with average r.m.s.d. of ~1.5 Å. The largest r.m.s.d. reached specific values of 2.9 and 3.2 Å at peptide positions P4 and P6, respectively. Although the r.m.s.d. for most peptide residues exhibit some overlap between SL9 variants and other peptides, those for SL9 at P4 and P7 are clearly separated from the others. These data indicate that the conformation common to all SL9 variants is distinct from that in other peptides bound to HLA-A2.
Basis for the Unique SL9 Peptide Conformation and Consequences for TCR Presentation—The SL9 conformation is notably distinct from other HLA-A2-restricted peptides at the P4 and P5 positions, but the fully exposed side chains at these positions obviously do not determine the conformation directly, and hydrophobic substitutions are accommodated without structural change (supplemental Table 1S). The partially buried side chains at P3, P6, and P7 must therefore be important determinants of conformation. Nevertheless, we find the same conformation with Ala replacing P3 Tyr and with Ile or Ala replacing P6 Val, and the structure presents no obvious impediment with Ser or Thr replacing P7 Ala, variants that are known to bind HLA-A2 (2). It seems that a balance of multiple effects conspire to maintain the unique conformation of SL9 variants in complex with HLA-A2.

The special conformation at P4 and P5 generates hydrogen bonding opportunities that are particular to the presentation of SL9. For example, the \( \phi/\psi \) angles at P4 are uniquely \( \alpha \)-like so that this carbonyl oxygen points toward HLA helix \( \alpha_1 \) and could be an acceptor from a TCR hydrogen bond. Thus, conservation of the SL9 conformation among SL9 variants preserves any backbone-directed interactions that TCRs may use in recognizing these pMHC complexes.

Analysis of the SL9 Peptide in the Context of the p17 Structure—To understand the relative lack of natural variation of residues critical for TCR recognition, we analyzed the SL9 peptide in the context of the structure of the HIV matrix protein p17 (31). The SL9 peptide is located within a central, largely buried \( \alpha \)-helix (helix 4) about which the p17 structure is organized, making hydrophobic contacts to all other secondary structure elements. Residues that correspond to the SL9 segment form part of this hydrophobic core. P2 Leu, P6 Val, P8 Val, and P9 Leu are completely buried, and P3 Tyr is squeezed between three Leu and one Ile (residues 61, 64, and 68 in helix 4 and residue 101 in helix 6), locking helices four, five, and six together (Fig. 6). P7 Ala points in the direction of P3 Tyr and is thereby constrained in its size. The importance of the SL9 segment in maintaining the structural integrity of p17 provides an explanation for the relative conservation of the region. P5 Thr forms the boundaries to the hydrophobic core; therefore, this residue may be naturally mutated into an Ala or Leu (see Table 1). The reason for complete conservation of P4 Asn in all known HIV and simian immunodeficiency virus isolates is not obvious from the p17 structure. Thus, the location of SL9 in situ in p17 places certain constraints on its sequence, and there may be additional constraints from other uses of the gene.

DISCUSSION

Every TCR expressed on peripheral T cells is selected in the thymus as a result of its productive engagement by self- or syn-genic MHC (synMHC) in association with peptides derived from normal intracellular proteins (32–34). Mature peripheral T cells usually do not respond to such self-pMHC complexes, but they could vigorously react to a synMHC-bound peptide derived from a protein that is not normally produced in the host body. In addition, T cells recognize disparate MHC molecules from other individuals of the same species, termed allogeneic MHC (35). Therefore, it appears that every TCR could recognize multiple distinct pMHC complexes. It has been estimated that the number of different ligands seen by the same TCR could be as high as \( 10^6 \) (36).

How can a single TCR bind “specifically” to different pMHC ligands? One possibility is that the TCR adjusts its conformation in order to achieve optimal binding to several different
pMHCs. It has been proposed that the TCR contact surface is flexible and undergoes conformational changes upon binding to a cognate pMHC complex (37–40). Another mechanism, which invokes the contribution of solvent to TCR specificity, has been also proposed (4). Because conformational changes in the TCR structure that permit recognition of various pMHCs are limited, the following question arises: what is the extent of variations in the recognizable surface of pMHC complexes that can be accommodated by a TCR?

The issue of accommodation to pMHC variation is particularly relevant to recognition of virus epitopes by T cells, an essential modality for optimizing new generation vaccines (41). Many viruses, notably HIV, undergo mutations leading to changes in their proteins. These changes often occur within T cell epitopes and, consequently, may lead to escape from immune surveillance (42). Such changes do not necessarily preclude epitope recognition and the persistence of T cell responses, however (2, 43).

Based on our high resolution crystal structures and binding data, we suggest that degeneracy in recognition of SL9-HLA-A2 by D3 TCR is accommodated by preserving a common mode of binding for all MHC-bound SL9 variants. We found that all tested peptide variants assume nearly identical conformations upon binding to HLA-A2 (Fig. 4a) despite up to three amino acid changes in the nine-residue epitope. This conformation, which is distinct from that of other HLA-A2-bound peptides, is such that it presents several backbone-directed hydrogen-bonding potentials. There is sensitivity to natural substitutions at P5 (Table 1), which is

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FIGURE 4. Comparison of SL9 and its variants as bound to HLA-A2. a, top view of the overlapping peptides as they are bound to the HLA-A2 molecule. The peptides are SL9, SL9-3A, SL9-3F/6I/8V, SL9-4L, SL9-5V, SL9-6I, and SL9-1A6A8A (see Table 1). Carbon atoms are colored in yellow; oxygen atoms are in red, and nitrogen atoms are in blue; bonds are in yellow except for N-Cα bonds in blue. b, side view of the overlapping peptides, as in a but rotated 90°. A remarkably similar peptide conformation is assumed by all peptides upon binding to HLA-A2. Variation in side-chain conformations reflects intrinsic freedom as sampled in the simulated annealing refinement. c, side view comparison of the SL9 peptide (tyrosine at P3) with the 3A-SL9 peptide (alanine at P3) (3A-SL9 at P3). Models are oriented as in b, but SL9 is now colored in red and 3A-SL9 is colored in blue. d, fractional solvent accessibility of SL9 side chains as bound to HLA-A2. The solvent-accessible surface area for side-chain atoms (including Cα/H) of the SL9 peptide bound to HLA-A2 is compared at each position with that in the isolated peptide with side chains neighboring the specified position removed, i.e. for the Gly-X-Gly peptide.

FIGURE 5. Positional deviations between SL9 and other nonameric peptides bound to HLA-A2. r.m.s.d. for backbone atoms including Cα/H are recorded at each peptide position for each comparison. Blue horizontal marks compare SL9 with unrelated nonameric peptides bound to HLA-A2 in crystal structures deposited as Protein Data Bank entries 1i1f, 1a07, 1i7r, 1i7t, 1i7u, 1akj, 1b0g, 1bd2, 1duz, 1hhg, 1hhj, 1hhk, and 1jht. Red horizontal marks are for comparisons between SL9 and SL9 variants. Blue and red lines connect the average deviations for the respective groups.

FIGURE 6. Structure of the SL9 peptide embedded in the HIV matrix protein p17. The p17 structure is represented as a ribbon diagram on a blue background with the segment within helix 5 that corresponds to SL9 colored orange. Side chains of SL9 residues P3 Tyr and P7 Ala are drawn with carbon atoms in gray and the tyrosyl oxygen atom in red. Residues making hydrophobic contact with P3 Tyr located in helixes 4 and 6 are in yellow.

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Recent studies describe a distinct conformation for the natural SL9 variant SL9-3F (44). This peptide binds with 20-fold lower affinity to HLA-A2 than SL9 (Table 1) but is equally active in the CTL assay (Table 1). Because binding of SL9- and SL9-3F-HLA-A2 complexes to TCR is characterized by similar kinetics and thermodynamics (44), it is presupposed that SL9 and SL9-3F assume similar conformations upon recognition by the TCR (44), further emphasizing the significance of our findings. Others have reported recently that the unique conformation of a peptide from Epstein-Barr virus bound to one but not another MHC allele is responsible for peptide immunogenicity (45) providing additional evidence of the critical role of the MHC-I-bound peptide conformation in recognition by TCR.

Natural variation in the SL9 peptide is intrinsically restricted by the critical placement of this peptide in the Gag protein p17. Indeed, some mutations in the SL9 segment of p17 lead to disruption of the p17 structure and consequently influence the assembly and budding of the virus (46). The structural constraints imposed by requirements of the p17 structure may also in turn preserve the peptide conformation of variants as bound to HLA-A2. Degenerate recognition of SL9 by D3 TCR exceeds this imposed conservation; however, synthetic variants that replace otherwise invariant P4 Asn with Gln or Leu are still recognized by D3 TCR (Table 1).

The mechanism of degenerate recognition of the HIV Gag epitope described here appears to be very different from that recently unraveled for recognition of influenza virus peptide from the nuclear protein (47), a common epitope for all influenza virus strains. It has been proposed that a highly optimized TCR-pMHC pairing dominates the CTL response against influenza, which is very different from recognition of SL9-HLA-A2 during chronic HIV infection where several CTL clones perform comparably well (2). Thus, two different mechanisms of degenerate recognition of nuclear protein and SL9 pMHC complexes are likely to contribute to immunodominant CTL response against these viral epitopes.

Because the described degeneracy in recognition of SL9 is principally determined by structural constraints necessary for virus survival, persistence of HIV-specific CTL responses may rather depend on the nature of the antigen and to a lesser extent on the repertoire of T cells in infected people. This emphasizes the potential importance for immunodominant peptide epitopes as vaccine candidates. Unfortunately, natural viral proteins do not always make optimal vaccines (48). In fact, a p17 Gag containing Canarypox vaccine failed to elicit SL9-specific CTL in uninfected individuals (49) consistent with the usual absence of such CTL during the acute phase of the infection. Because the majority of HLA-A2-positive adults with chronic HIV infection have a detectable response directed against SL9, inefficient presentation of this peptide during vaccination and acute phase of infection is thought to be responsible for the absence of SL9-specific CTL. The strategy of “epitope enhancement” (50) in new generation vaccines might help to elicit a desirable response of HIV-specific CD4 and CD8 T cells, including anti-SL9 CTL, to enhance protective immunity against HIV infection.

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