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*J Immunol* 2011; 186:2950-2958; Prepublished online 31 January 2011;
doi: 10.4049/jimmunol.1003150
http://www.jimmunol.org/content/186/5/2950

Supplementary Material  http://www.jimmunol.org/content/suppl/2011/01/31/jimmunol.1003150.DC1

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Conformational Melding Permits a Conserved Binding Geometry in TCR Recognition of Foreign and Self Molecular Mimics

Oleg Y. Borbulevych,* Kurt H. Piepenbrink,* and Brian M. Baker*†

Molecular mimicry between foreign and self Ags is a mechanism of TCR cross-reactivity and is thought to contribute to the development of autoimmunity. The αβ TCR A6 recognizes the foreign Ag Tax from the human T cell leukemia virus-1 when presented by the class I MHC HLA-A2. In a possible link with the autoimmune disease human T cell leukemia virus-1–associated myelopathy/tropical spastic paraparesis, A6 also recognizes a self peptide from the neuronal protein HuD in the context of HLA-A2. We found in our study that the complexes of the HuD and Tax epitopes with HLA-A2 are close but imperfect structural mimics and that in contrast with other recent structures of TCRs with self Ags, A6 engages the HuD Ag with the same traditional binding mode used to engage Tax. Although peptide and MHC conformational changes are needed for recognition of HuD but not Tax and the difference of a single hydroxyl triggers an altered TCR loop conformation, TCR affinity toward HuD is still within the range believed to result in negative selection. Probing further, we found that the HuD–HLA-A2 complex is only weakly stable. Overall, these findings help clarify how molecular mimicry can drive self/nonself cross-reactivity and illustrate how low peptide-MHC stability can permit the survival of T cells expressing self-reactive TCRs that nonetheless bind with a traditional binding mode. The Journal of Immunology, 2011, 186: 2950–2958.

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Received for publication September 21, 2010. Accepted for publication December 5, 2010.

This work was supported by the National Institute of General Medical Sciences, National Institutes of Health (GM067079); the Notre Dame Chemistry/Biochemistry/Biology Interface training program funded by the National Institute of General Medical Sciences, National Institutes of Health (GM075762 to K.H.P.); and by a fellowship from the Walther Cancer Foundation (to O.Y.B.). Results shown in this report are derived from work performed at the Structural Biology Center, Lilly Research Laboratories Collaborative Access Team, and Life Science Collaborative Access Team at the Advanced Photon Source, Argonne National Laboratory. Argonne is operated by UChicago Argonne for the U.S. Department of Energy under contract DE-AC02-06CH11357. Use of the Lilly Research Laboratories Collaborative Access Team facilities at Sector 31 of the Advanced Photon Source was provided by Eli Lilly & Company, which operates the facility.

The coordinates and structures presented in this article have been submitted to the Brookhaven Protein Data Bank under accession numbers 3PWL, 3PWP, 3PWN, and 3PWJ.

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The online version of this article contains supplemental material.

Abbreviations used in this article: HAM/TSP, human T cell leukemia virus-1–associated myelopathy/tropical spastic paraparesis; HTLV-1, human T cell leukemia virus-1; PDB, Brookhaven Protein Data Bank; pMHC, peptide-MHC; RMSD, root mean square deviation.

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.1003150
microbial Ag recapitulated the unusual binding mode seen with the myelin basic protein self Ag (16). In another demonstration, the murine TCR 2C cross-reacts with the foreign and self SIYR and dEV8 Ags via a common, traditional binding mode (17).

Yet differential docking of a single TCR on foreign and self pMHC ligands can occur, as demonstrated by the different orientation 2C adopts on the foreign QL9/H-2Ld ligand (18). Although this latter case is not one of molecular mimicry, unanticipated complexities observed in recent structural analyses of molecular mimics, including TCR, peptide, and MHC conformational changes and poor translation of sequence into structural mimicry (13, 14), warrants caution when presuming that foreign and self mimics will be recognized similarly.

In this study, we examined cross-recognition between self and nonself molecular mimics by the A6 TCR. As noted above, A6 recognizes the HTLV-1 Tax Ag presented by HLA-A2 (positions 11–19 of the Tax protein; sequence LLFGYPVVY). Although it is not highly promiscuous (19), A6 cross-reacts with a variety of modified Tax variants that share previously identified key residues, as well as microbial peptides, such as the yeast Tel1p peptide (13, 19–22). In a possible link with autoimmunity, A6 also recognizes a self Ag from an immunodominant region of the human neuronal protein HuD (positions 87–95; sequence LGYGFVNYI) (21, 23).

In this study, we examined cross-recognition between self and nonself molecular mimics by the A6 TCR. As noted above, A6 recognizes the HTLV-1 Tax Ag presented by HLA-A2 (positions 11–19 of the Tax protein; sequence LLFGYPVVY). Although it is not highly promiscuous (19), A6 cross-reacts with a variety of modified Tax variants that share previously identified key residues, as well as microbial peptides, such as the yeast Tel1p peptide (13, 19–22). In a possible link with autoimmunity, A6 also recognizes a self Ag from an immunodominant region of the human neuronal protein HuD (positions 87–95; sequence LGYGFVNYI) (21, 23). T cell recognition of epitopes from HuD have been suggested to play a role in the neurologic disorder human T cell leukemia virus-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), a demyelinating autoimmune disease affecting a minority of HTLV-1–infected individuals (24, 25). Patients with HAM/TSP that are HLA-A2–positive have persistent, high levels of CD8+ T cells specific for the Tax epitope in their peripheral blood and cerebral spinal fluid (26, 27), and the A6 T cell clone was isolated from a HAM/TSP-affected individual (28). The Tax and HuD peptides have related sequences (Fig. 1A), sharing a key loop. The structural changes contributed to a large, 70-fold weaker affinity of A6 for the HuD ligand. However, TCR affinity toward HuD was still within the range shown to result in negative selection in other systems (29). Probing further, we found that the HuD–HLA-A2 complex is only weakly stable, explaining why HuD-reactive T cells can be found in the periphery despite the existence of T cells that engage them with the traditional anti-foreign binding mode.

Materials and Methods

Proteins and peptides

Soluble HLA-A2 and A6 TCR were refolded from bacterially expressed inclusion bodies as previously described (30). Peptides were synthesized locally or purchased from GenScript; peptide purity and identity were confirmed by liquid chromatography/mass spectrometry. Streptavidin was purchased from Rockland. The A6 TCR used included an engineered disulfide bond across the constant domains for improved heterodimer stability (31).

Crystallization and x-ray analysis

Peptide–HLA-A2 crystals were grown from 24% PEG 3350 in 25 mM MES, pH 6.5, 0.1 M NaF or 0.1 M NaCl. A6–HuD–HLA-A2 crystals were grown from 15% PEG 4000 in 0.1 M Tris, pH 8.5, 0.2 M MgCl2. Cryoprotection for all crystals consisted of 20–25% glycerol. Diffraction data were collected at Argonne National Laboratory at the indicated beamlines at 100 K using a wavelength of 0.979 Â. Data reduction, structure solution, refinement, and structure validation were performed as previously described (19). Coordinates for search models for the pMHC structures were from Brookhaven Protein Data Bank (PDB) entries ITTV (32) for pMHC and 2GJ6 (19) for TCR–pMHC. Data collection and refinement statistics are presented in Table I. Surface area analysis was performed with the program Surface Racer using a probe radius of 1.4 Å (33). Shape complementarity (34) was calculated as implemented in CCP4 (35). Coordinates and structure factors are available from the PDB (www.rcsb.org). PDB accession numbers are 3PWL for HuD–HLA-A2, 3PWP for A6–HuD–HLA–A2, 3PWN for HuDG2L–HLA–A2, and 3PWJ for HuD_{G2L,I9V}–HLA–A2.

Surface plasmon resonance

Surface plasmon resonance measurements were performed with a Biacore 3000 instrument as previously described (30). All data were collected at 25°C. Solution conditions were 10 mM HEPES, 3 mM EDTA, 150 mM NaCl, 0.005% surfactant P-20, pH 7.4. pMHC complexes were coupled to the sensor surface via a biotin–streptavidin linkage, with the streptavidin amine coupled to a CM5 sensor chip. Biotinylated HuD–HLA–A2, HuD_{G2L–A2}, and HuD_{G2L–I9V–HLA–A2} were coupled to three of the flow cells, with the fourth serving as a reference. Soluble A6 TCR was then injected at 5 μl/min until steady state was reached. Each injection series was repeated twice and the data globally analyzed. Data were processed in BIAevaluation 4.1 and analyzed with Origin 7.5.

Thermal stability

Circular dichroism measurements of pMHC thermal stability were performed using a Jasco J-815 spectrometer monitoring 218 nm. Solution conditions were 20 mM phosphate and 75 mM NaCl (pH 7.4). Sample concentrations were 5 μM. A temperature increment of 1°C/min was used, maintaining the temperature within 0.1°C for 5 s before sampling. As peptide–HLA–A2 complexes do not show reversible unfolding, the data were fit to a nine-order polynomial and the apparent Tm taken from the maximum of the first derivative of the fitted curve.

Results

The HuD–HLA-A2 self-complex is a close but imperfect mimic of the Tax–HLA-A2 nonself complex

We first determined the structure of the HuD–HLA–A2 complex at 1.65 Å resolution (Table I). The complex displayed the usual class I pMHC architecture with two molecules per asymmetric unit (Fig. 1A). There are no substantial differences between the two molecules in the unit cell (all atoms of the two peptides superimpose with an root mean square deviation (RMSD) of 0.1 Å; the backbones of the HLA–A2 peptide binding domains superimpose with an RMSD of 0.4 Å). Electron density images for the peptides in the HLA–A2 peptide binding grooves appear in Supplemental Fig. 1.

Comparison of the HuD–HLA–A2 complex with the Tax–HLA–A2 complex (36) revealed the HuD complex to be a close but imperfect mimic of the Tax complex. Other than a small region of the α2 helix discussed below, the HLA–A2 H chains are in full alignment (Fig. 1B). The peptide backbones adopt the same conformation, superimposing with an 0.4 Å RMSD (Fig. 1C). The majority of the peptide side chains overlay each other. However, the side chain of Tyr3 of HuD is rotated 110° compared with its analogue Phe3 in Tax, pointing the side chain toward the base of the peptide binding groove. Likewise, the side chain of Phe5 is rotated 120° compared with its analogue Tyr5 in Tax, also pointing the side chain toward the base of the binding groove. The concerted difference in the positions 3 and 5 side chains is attributable

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to differences in β-stacking between the two aromatic side chains: in HuD, Tyr3 and Phe5 are arranged in a classic parallel-displaced arrangement, whereas in Tax they are arranged in a T-shaped fashion (37). The differences reflect a common structural and energetic link between nonadjacent peptide side chains in pMHC complexes (38) and alter how an incoming TCR would see the peptide, particularly at the position 5 aa, which in the complex of the A6 TCR with the Tax peptide is accommodated in a central pocket formed by the CDR3α and CDR3β loops (4).

The small “linker region” connecting the short and long helical elements of the α2 helix in the HuD–HLA-A2 complex is slightly shifted relative to its position in Tax–HLA-A2 (highlighted in Fig. 1B; electron density images for this region are shown in Supplemental Fig. 3). Peptide-dependent shifts have been noted in this region previously (e.g., see Ref. 39), likely reflecting an influence of the peptide on HLA-A2 flexibility as recently described with the yeast Tel1p peptide (13). The shift is small, only 1.1 Å at the α carbon of Ala150. However, as discussed below, small changes in

Table I. X-ray data collection and refinement statistics

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*Values in parentheses are for highest-resolution shell.

APS, advanced photon source; RMS, root mean square.

FIGURE 1. Tax and HuD complexes with HLA-A2 are close but imperfect mimics. A. Sequences of the Tax and HuD peptides. The conserved amino acids at positions 2, 4, and 8 are in red, and the homologous tyrosine/phenylalanine aromatics at positions 3 and 5 are in blue. B. Overview of the peptides and peptide binding grooves in the Tax–HLA-A2 and HuD–HLA-A2 complexes. The Tax–HLA-A2 complex is pink, and the HuD–HLA-A2 complex is light blue. Superimposition is through the backbones of aa 1–180 of the HLA-A2 H chains and all nine atoms of the peptides. The circle highlights a small shift in the α2 helix “linker region.” The shift is maximal at the α carbon of Ala150, as shown in the backbone trace below the ribbon diagram. C. Cross-eyed stereo view of the peptides from the superimposition in B. The backbones of the two peptides are identical. The only significant side-chain differences occur at the side chains of positions 3 and 5 (phenylalanine and tyrosine in Tax, tyrosine and phenylalanine in HuD).
this region impact TCR recognition of the HuD–HLA-A2 complex.

The A6–HuD–HLA-A2 ternary complex is very similar to the A6–Tax–HLA-A2 complex

We next determined the structure of the ternary complex of the A6 TCR bound to HuD–HLA-A2 at a resolution of 2.7 Å (Table I; see Supplemental Fig. 2 for electron density images). The complex is very similar to the ternary complex of A6 with the Tax peptide, with the TCR positioning itself over HuD–HLA-A2 exactly as it does over Tax–HLA-A2, forming the same docking angle and tilt (Fig. 2A). With the exception of CDR3β, which is shifted toward the periphery of the interface as discussed later, the loops of A6 are in the same conformation in both complexes (Fig. 2B). A6 recognition of the Tax and HuD foreign and self Ags thus proceeds with the same traditional binding mode.

FIGURE 2. A6 TCR engages the HuD–HLA-A2 ligand identically to how it engages the Tax–HLA-A2 ligand, with a reorganization of the CDR3β loop and a small shift in the α2 helix linker region. A, Overall comparison of the A6–HuD–HLA-A2 and A6–Tax–HLA-A2 complexes, showing the TCR variable domains, peptides, and the HLA-A2 peptide binding domain. The coloring scheme is indicated below the figure and is maintained in all panels. Superimposition is through the backbones of the TCR variable domains, peptides, and HLA-A2 peptide binding domain. B, Top view of the superimposed TCR–pMHC complexes, showing the CDR loops over the pMHC molecules. Other than CDR3β, the CDR loops are in the same position in the HuD and Tax complexes. C, Cross-eyed stereo view showing the differential positioning of CDR3β in the HuD and Tax complexes. The α carbons of Gly101 and Pro103 shift by 3.3 and 2.0 Å, respectively. D, The linker region of the HLA-A2 α2 helix is displaced away from the peptide upon recognition of HuD. Measured at the α carbon of Ala150, the displacement is 1.4 Å.
The difference in the TCR CDR3β loop between the HuD and Tax complexes is maximal at the α carbon of Gly101β, which in the HuD complex is moved toward the periphery of the interface by 3.3 Å (Fig. 2C). Further along the loop, Pro103β is displaced by 2.0 Å. Although the differences in the loop between the two structures are structurally significant, in context they are not unusual, as the maximal 3.3 Å shift is just below the average CDR3β movement tabulated in a recent comparison of free and bound TCRs (40). There is no obvious steric reason for the shift in CDR3β. However, CDR3β in A6 has been found to adopt a range of conformations in various structures with variants and mimics of the Tax peptide (13, 19, 20, 41). As discussed later, the loop shift reflects a need to optimize interface electrostatics, triggered by the loss of a hydrogen bond between position 5 of the peptide and the TCR.

The shift in the α2 helix linker region noted in the structure of the free HuD–HLA-A2 complex is more pronounced in the A6–HuD–HLA-A2 complex (Fig. 2D), with the region backing away from its position in the A6–Tax–HLA-A2 complex by 1.4 Å as measured at the α carbon of Ala150. Although smaller and less of a reorganization than that seen for CDR3β, movement of the α2 helix impacts the contacts made across the interface, likely contributing to the weaker affinity A6 maintains toward HuD–HLA-A2.

Although the TCR binds with the same mode, the amino acid differences between Tax and HuD, the different CDR3β conformation, and the small shift in the α2 helix linker region lead to a number of altered contacts within the TCR–pMHC interface. The majority of the differences are between CDR3β, the center of the peptide, and the shifted region of the HLA-A2 α2 helix. There is a net loss of six hydrogen bonds or salt-bridges in the HuD structure. Despite the changes, with few exceptions the pattern of amino acids used to form the complex is similar in Tax and HuD. The “hotspot” residue of Arg65 of the HLA-A2 α2 helix remains a major participant (42, 43), as do the other amino acids comprising the proposed class I MHC “restriction triad” (Arg65, Ala69, and Gln155 in HLA-A2) (44). A detailed comparison of the structural features of the HuD and Tax TCR–pMHC interfaces is provided in Supplemental Fig. 4.

Are there features in the A6–HuD–HLA-A2 interface easily identifiable as “suboptimal”? As noted above, the shift in CDR3β is within the range of those seen previously. The amount of buried solvent-accessible surface area in the HuD and Tax interfaces is nearly identical (2084 Å² with Tax versus 2128 Å² with HuD), as is the proportion of buried hydrophobic/hydrophilic surface (54% hydrophobic with Tax, 55% hydrophobic with HuD). The total number of interatomic contacts is also nearly identical (130 with Tax versus 126 with HuD). Although the number of hydrogen bonds and salt-bridges is reduced in the HuD complex compared with that of the Tax complex (15 with Tax, 9 with HuD), the number present is still within the range seen in other TCR–pMHC complexes with foreign Ags (45). Although the contacts are altered, the general pattern of amino acids contacted on both sides of the interface is similar with both the HuD and Tax peptides (see Supplemental Fig. 4). Lastly, the shape complementarity in the A6–HuD–HLA-A2 interface is actually improved compared with that in the A6–Tax–HLA-A2 interface (0.66 with HuD versus 0.63 with Tax). Thus, a priori, the complex of A6 with HuD–HLA-A2 does not show any easily identifiable “suboptimal” features.

The shift in CDR3β is attributable to imperfect chemical mimicry between HuD and Tax

Although the CDR3β loop is in a different conformation in the HuD structure than in the Tax structure, we noticed that the conformation of the loop is very similar to an alternate conformation observed previously with a variant of the Tax peptide. This peptide, referred to as TaxYSF(3,4FF), replaced Tyr5 of Tax with a doubly fluorinated phenylalanine derivative, with one of the two fluorines substituting for the tyrosine hydroxyl (41) (Fig. 3A). The fact that both structures showed nearly the same altered loop conformation suggested to us a mechanism in which the conformation of the CDR3β loop is dependent upon electrostatic interactions in the interface.

Notably, the HuD and the TaxYSF(3,4FF) peptides alter interface electrostatics similarly. In the structure with the native Tax peptide, the side chain of Arg95 of CDR3β, at the apex of the pocket formed by the CDR3α and CDR3β loops, hydrogen bonds to the Tax Tyr5 hydroxyl (Fig. 3B). Neither the HuD peptide nor the TaxYSF(3,4FF) peptide can form this hydrogen bond [fluorine is a poor hydrogen bond acceptor (46)], and in both cases Arg95β moves away from the side chain (Fig. 3C, 3D). The loss of this hydrogen bond in both structures is countered by the formation of a new salt-bridge between Arg102β seven residues down the loop and Gln154 of the HLA-A2 H chain. The difference in the position of the CDR3β loop in the HuD and TaxYSF(3,4FF) complexes thus results from the need to optimize electrostatics between the TCR and the pMHC, stemming from the loss of the

![FIGURE 3. Altered conformation of the CDR3β loop in the A6–HuD–HLA-A2 complex results from the loss of a single hydrogen bond and the subsequent need to reoptimize interface electrostatics. A, Comparison of CDR3β from the Tax, HuD, and TaxYSF(3,4FF) complexes, generated through superimposition of the backbones of the TCR Vα/Vβ domains. B, In the Tax complex, Tyr5 of the peptide hydrogen bonds to Arg95 of CDR3β. There is no interaction between Arg102β and Gln154 of the HLA–A2 H chain. C, In the HuD complex, there is no hydrogen bond to Arg95 of CDR3β. To compensate, the reorganized loop forms a salt-bridge between Arg102β and Gln154 of the HLA–A2 H chain. D, In the TaxYSF(3,4FF) complex, the hydrogen bond to Arg95β is also lost, and the reorganized loop forms a similar salt-bridge with Gln154 of the H chain.](http://www.jimmunol.org/)

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hydrogen bond to the position 5 side chain. In other words, the difference of a single hydroxyl appears to trigger the altered positioning of the CDR3β loop.

**Peptide conformational changes in HuD but not Tax upon A6 binding contribute to a weaker TCR affinity**

In the complex with the TCR, the HuD peptide adopts the same conformation as the Tax peptide, as is clear in Fig. 2. As the side chains of Tyr3 and Phe5 of the HuD peptide are presented by HLA-A2 in conformations different from their counterparts in the Tax peptide, they must therefore rotate in order for the ternary complex to form (Fig. 4A, 4B). Similar conformational changes are not needed for A6 recognition of the Tax peptide, as the side chains of Phe3 and Tyr5 are presented in conformations compatible with TCR recognition (Fig. 4C, 4D).

With glycine at position 2 and isoleucine at position 9, the HuD peptide has poor anchor residues for HLA-A2 (47). We were initially concerned that reduced stability of the pMHC complex would impede crystallization and thus studied anchor-modified variants of the HuD peptide. In the first variant, glycine 2 was substituted with leucine (HuDG2L). The second variant included variants of the HuD peptide. In the first variant, glycine 2 was substituted with valine (HuDG2L/Val). Although as demonstrated earlier, reduced pMHC stability did not impact our studies with the native peptide, we did crystallize and determine the structures of the complexes of the two anchor-modified peptides with HLA-A2 (Table I; see Supplemental Fig. 3 for electron density images). Both complexes were identical to the complex with the native peptide except in the positioning of the side chains of Tyr3 and Phe5. Unlike the native peptide, in the HuDG2L–HLA-A2 complex, the peptide was presented with both side chains mimicking their conformations in the A6–HuD–HLA-A2 complex (this was true for both molecules in the asymmetric unit, for which all atoms of the peptide superimposed with an RMSD of 0.2 Å) (Fig. 5A). The positioning of the side chains in the HuDG2L/Val–HLA-A2 structure was more complicated: the Tyr3 and Phe5 side chains in the first molecule in the asymmetric unit adopted their TCR-bound conformations, but the electron density in the second molecule in the asymmetric unit allowed the side chains to be modeled in both the TCR-bound and TCR-free conformations of the native peptide (Fig. 5B). Anchor modification thus perturbed the side-chain conformational equilibrium toward the TCR-bound state, with a greater perturbation in the singly modified HuDG2L peptide. The mechanism behind the perturbations is not clear, as there are no backbone or side-chain changes propagated from the anchor residues, and there are no crystallographic contacts in any of the structures that could influence side-chain position. However, anchor modification of class I MHC-presented peptides has previously been shown to affect the conformation of amino acids distant from the site of substitution (48–50).

A prediction from these structural results is that the affinity of A6 should be stronger for the anchor-modified HuD ligands than it is for the native ligand. To confirm this, we examined the affinity of the A6 TCR toward the native and the two anchor-modified HuD ligands in a Biacore binding assay. TCR affinity for the native ligand was weak at 140 μM, in good agreement with a previously reported measurement (23). Fully consistent with the structural data, the affinity for the doubly modified HuDG2L/Val variant was 2-fold stronger at 70 μM, whereas affinity for the singly modified HuDG2L variant was stronger still at 48 μM (Fig. 5C). For comparison, the affinity for the Tax ligand is a much stronger 2 μM (30).

The results with the anchor-modified peptides can be extrapolated to help explain the difference in the A6 TCR’s affinity toward the native HuD and Tax ligands. As A6 presents its central side chains in their TCR-bound conformation whereas HuD does not, we can conclude that one reason why A6 recognizes HuD more weakly than it does Tax is that, unlike Tax, the central aromatic side chains of the HuD peptide must shift into a binding-competent conformation in order for recognition to proceed.

The HuD–HLA-A2 complex is of low stability relative to the Tax–HLA-A2 complex

Although the A6 TCR recognized the HuD–HLA-A2 complex with a weaker affinity than that for the Tax–HLA-A2 complex, the affinity of 140 μM is still within a range believed to result in negative selection (29). As poor Ag presentation levels can also lead to escape from negative selection (51) and the HuD peptide has suboptimal anchors for HLA-A2, we examined the thermal stability of the HuD–HLA-A2 complex using circular dichroism spectroscopy. Circular dichroism has long been used to examine pMHC stability, and measurements of thermal stability correlate with pMHC binding affinity (52). The Tm of the native HuD–HLA-A2 complex was measured as 40°C (Fig. 6). As expected, anchor modification of the HuD peptide enhanced stability, with the Tm of the doubly modified HuDG2L/Val complex measured as 59°C and the Tm of the singly modified HuDG2L complex measured as 63°C. For comparison, the Tm of the Tax–HLA-A2 complex was measured as 62°C, in close agreement with previous studies (13, 36). Thus, the native HuD–HLA-A2 complex has exceedingly low stability relative to the Tax–HLA-A2 complex.

**FIGURE 4.** Side-chain conformational changes occur in A6 recognition of HuD but not Tax. A, Superimposed HuD peptides from the HuD–HLA-A2 and A6–HuD–HLA-A2 complexes, showing the 100° rotation of Tyr3 that occurs upon TCR binding. The peptide from the pMHC complex is light gray, and the peptide from the TCR–pMHC complex is dark gray; the arrow indicates the direction of rotation. B, Same as in A, but showing the 115° rotation of Phe5 that occurs upon TCR binding. C and D, Superimposed Tax peptides from the Tax–HLA-A2 and A6–Tax–HLA-A2 complexes, showing that unlike HuD, rotations in the positions of Phe3 (C) and Tyr5 (D) are not needed for TCR recognition. Peptides from the pMHC complex are light gray, and peptides from the TCR–pMHC complex are dark gray.

**Discussion**

T cell cross-reactivity between foreign and self Ags has been implicated in a number of autoimmune pathologies, and molecular mimicry between self and nonself is frequently discussed as an underlying component. Yet structures of TCRs bound to self Ags implicated in autoimmune have shown unusual features that distinguish them from structures of TCRs bound to foreign Ags (5–8). These observations have led to hypotheses that relate unusual TCR binding to reduced TCR affinity and T cell escape from negative selection, permitting the development of autoimmunity (11, 12). The extent to which unusual binding would
apply in the context of molecular mimicry however is unclear: unusual recognition of a self Ag together with more traditional recognition of a foreign Ag by the same TCR is inconsistent with molecular mimicry as traditionally envisioned (i.e., the conservation of key structural or chemical features translating into a similar mode of recognition). Although it is possible that conservation of “hotspots” could still permit differential TCR binding, resembling to some extent how the 2C TCR cross-reacts with the QL9/H-2Ld and dEV8/H-2Kb ligands (18), this still stretches the expectations of a molecular mimicry mechanism.

Cross-reactivity by the A6 TCR between the foreign Ag Tax and the self Ag HuD provided us with an opportunity to examine the structural basis for self/nonself cross-reactivity within the context of molecular mimicry. Consistent with a molecular mimicry mechanism, HLA-A2 presents the Tax and HuD peptides in very similar conformations, with differences in the positions of only two side chains. Also consistent with molecular mimicry, the A6 TCR engages HuD–HLA-A2 with the same center-focused, diagonal binding mode it uses to engage Tax–HLA-A2, with no differences in position or orientation. A traditional molecular mimicry mechanism in terms of both Ag presentation as well as receptor recognition can therefore account for TCR cross-reactivity between the Tax and HuD Ags.

Although the traditional anti-foreign TCR binding mode is conserved, there are differences in the interaction between A6 and the Tax and HuD complexes with HLA-A2. Unlike Tax, the central aromatic side chains of the HuD peptide must undergo conformational changes in order for the TCR to bind. There is a shift in the α2 helix that occurs upon recognition of HuD but not Tax. The inability of the central phenylalanine side chain of the HuD

**FIGURE 5.** Anchor modification in the HuD peptide reveals side-chain conformational differences as a component of weaker TCR affinity. A, The conformation of the peptide in the HuDG2L–HLA-A2 complex is identical to the conformation of the peptide in the ternary complex with the native, unmodified HuD peptide. The anchor-modified peptide is white, and the native peptide from the ternary complex is dark blue. B, The peptide in the first molecule in the asymmetric unit of the doubly modified G2L/I9V HuD–HLA-A2 complex adopts the conformation seen in the complex of the native peptide with the A6 TCR (top), whereas the peptide in the second molecule in the asymmetric unit could be refined in both the conformation seen when the native peptide is TCR-bound and when the native peptide is TCR-free (alternate positions in green). C, Surface plasmon resonance indicates that the affinity of the A6 TCR is higher for the anchor-modified peptide–HLA-A2 complexes than it is for the native HuD–HLA-A2 complex, consistent with the structural results showing that anchor modification biases the side-chain conformational equilibrium toward a binding-competent state.

**FIGURE 6.** The HuD–HLA-A2 complex is substantially less stable than the Tax complex as well as the anchor-modified HuDG2L and HuDG2L/I9V complexes as demonstrated by measurements of thermal stability monitored by circular dichroism spectroscopy. The solid lines represent polynomial fits to the data, and the apparent Tm values, taken from the first derivatives of the fitted curves, are indicated in the key.
peptide to form a hydrogen bond within the CDR3α/CDR3β pocket results in a repositioning of the CDR3β loop. It is thus only due to the structural plasticity available to both the TCR and the pMHC that A6 is able to cross-react between the two ligands. This conformational “melting” of both receptor and ligand is not unique to this case: A6 cross-reacts between Tax and its foreign mimic from the yeast Tel1p protein via similar, albeit larger, conformational changes in the TCR, peptide, and the MHC (13). The LC13 TCR cross-reacts between foreign peptides with related sequences but divergent structures by forcing them to adopt similar conformations in the TCR-bound state (14), and the CDR3α and CDR3β loops of LC13 undergo significant changes from their unbound forms (53, 54). Recognition of the pBM8/H‐ 
\^<sub>2km8</sub> ligand by the BM3.3 TCR occurs with changes in peptide and MHC, and although the structure of the free BM3.3 TCR is not available, the CDR3α and CDR3β loops adopt different conformations upon recognition of other ligands (55). To a lesser extent, conformational changes occur in foreign and self peptide/DR2β complexes upon recognition by the Ob.1A12, and again, there are differences in CDR loop positions in the foreign and self ternary complexes, and binding proceeds with kinetic and thermodynamic indicators of conformational changes (5, 16, 56).

Thus, although previous work highlighted the need to focus on structural as opposed to just sequence similarities when considering molecular mimicry (2, 3, 57), it is becoming increasingly clear that both ligand and receptor adaptability or flexibility and the resultant conformational melding often needed for binding add an additional layer of complexity.

Although CD8+ T cell recognition of HuD Ags has been implicated in HAM/TSP, and although the A6 TCR was cloned from a HAM/TSP patient, whether T cell recognition of cells presenting the HuD peptide is a component of disease is controversial. Nonetheless, HuD-specific T cells, such as those expressing A6, must still escape the process of negative selection. If low affinity stemming from an altered TCR binding mode cannot explain this, what mechanisms can? The structural melding needed for A6 engagement of HuD weakens affinity 70-fold compared with that for the Tax ligand. However, the affinity of ∼ 140 μM may not be sufficiently weak to allow escape, as TCR–pMHC interactions with affinities in this range can still result in negative selection (29). A more likely explanation is found in the low stability of the HuD–HLA-A2 complex: in addition to weak TCR binding affinities, low levels of Ag presentation can also permit T cell escape from negative selection (51), and poorly stable pMHC complexes require correspondingly higher affinity TCR affinities for productive signaling (58). The HuD self Ag thus provides an example of how weak peptide binding to MHC can permit the survival of T cells with receptors capable of recognizing self Ags with a traditional anti-foreign binding mode. Similar observations have been made with the 2C TCR (17), suggesting such behavior may be common. Thus, cross-recognition of weakly stable self mimics of foreign Ags via conserved, traditional binding modes likely contribute to the development and progression of autoimmunity.

Acknowledgments
We thank Cynthia Piepenbrink for outstanding technical assistance.

Disclosures
The authors have no financial conflicts of interest.

References

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Figure S1. Peptide 2Fo-Fc electron density in the native and anchor modified HuD/HLA-A2 structures. Mol1 (chain C) is for the first molecule in each asymmetric unit; Mol2 (chain F) is for the second molecule in each asymmetric unit.

Figure S2. Peptide (left) and CDR3/γ68/γ69 loop (right) 2Fo-Fc electron density in the A6-HuD/HLA-A2 structure.

Figure S3. 2Fo-Fc electron density for the α2 helix linker region in the free HuD/HLA-A2 complex (left; for Mol 1) and in the A6-HuD/HLA-A2 complex (center).
Figure S4. Comparison of the A6-peptide/HLA-A2 interfaces with Tax and HuD. A) Contacts to the TCR binding loops in the two interfaces. Loop sequence is shown across the center. The number of contacts to each amino acid is in blue, with Tax contacts above the sequence and HuD contacts below. HLA-A2 or peptide amino acids forming contacts are also shown, with the number of contacts given in parentheses. Yellow highlights indicate the amino acid participates in a single hydrogen bond or salt bridge; red highlights indicate two hydrogen bonds or salt bridges; and green indicates three hydrogen bonds or salt bridges. Loops are truncated at both ends for clarity. Superscripts on the first number of each loop sequence give the amino acid number according to the numbering in the A6-HuD/HLA-A2 PDB file. B) Number of contacts to amino acids in the HLA-A2 α1 and α2 helices and the peptides. Highlights are defined as in panel A. Contacts defined as interatomic distances ≤ 4 Å and include all backbone and sidechain atoms.