Molecular mimicry of self-epitopes by viral antigens is one possible pathogenic mechanism underlying induction of autoimmunity. A self-epitope, mDBM, derived from mouse dopamine β-mono-oxygenase (KALYDYAPI) sharing 44% sequence identity with the lymphocytic choriomeningitis virus-derived immunodominant epitope gp33 (KAVYNFATC/M), has previously been identified as a cross-reactive self-ligand, presentation of which results in autoimmunity. A rat peptide homologue, rDBM (KALYNAPI, 56% identity to gp33), which displayed similar properties to mDBM, has also been identified. We herein report the crystal structure of H-2Db-rDBM and a comparison with the crystal structures of the cross-reactive H-2Db-gp33 and non-cross-reactive H-2Db-gp33 (V3L) escape variant (KALYNFATM, 88% identity to gp33). Despite the large sequence disparity, rDBM and gp33 peptides are presented in nearly identical manners by H-2Db, with a striking juxtaposition of the central sections of both peptides from residues p3 to p7. The structural similarity provides H-2Db in complex with either a virus-derived or a dopamine β-mono-oxygenase-derived peptide with a shared antigenic identity that conserves the positioning of the heavy chain and peptide residues that interact with the T cell receptor (TCR). This stands in contrast to the T cell receptor (TCR). This stands in contrast to the T cell receptor (TCR) interactions with the TCR-interacting surfaces of the H-2Db-rDBM and H-2Db-gp33 major histocompatibility complexes are very similar with regard to shape, topology, and charge distribution, providing a structural basis for CD8 T cell activation by molecular mimicry and potential subsequent development of autoreactivity.

Antigen-specific CD8+ T cell responses are triggered by the interaction between a T cell receptor (TCR)1 and a cognate peptide-MHC class I complex. Although T cells are generally described to be specific for a foreign (e.g. viral) peptide bound to an MHC class I molecule, it is now clear that there is a degree of degenerate recognition of peptide-MHC class I complexes by TCRs (1). Thus, a TCR specific for a viral peptide-MHC class I complex can cross-react with multiple other peptide-MHC class I complexes, including antigen variants and self-peptides (2–12). The cross-reactive peptides can be unrelated in sequence, indicating that the cross-reactivity rather depends on similarities in the three-dimensional structures of the peptide-MHC class I complexes (2, 11, 13–15).

Molecular mimicry has been defined as similar structures shared by molecules encoded by dissimilar genes (16). The molecular mimicry hypothesis postulates that T cells specific for microbial mimicking epitopes can cross-react with MHC-restricted self-peptides. After an initial triggering and expansion of T cells by viral or bacterial peptide-MHC complexes, the T cells can cross-react with self-epitopes in the periphery, potentially triggering autoimmune responses (2, 11, 13–15, 17–22). The development of autoimmunity has been commonly associated with CD4+ T cells (13). However, there are now both clinical and experimental observations that suggest that auto-reactive CD8+ T cells may also be involved (23–26). Although autoreactivity may result from a cross-reactive immune response to foreign molecules that mimic self proteins (19), the importance of molecular mimicry in the development of autoimmune disease remains somewhat controversial (27).

Lymphocytic choriomeningitis virus (LCMV) infection of H-2b mice (e.g. C57BL/6 mice) generates a CD8+ CTL response mainly directed toward three immunodominant epitopes (28). More than 50% of the total LCMV-specific CTL activity is directed toward one such peptide, gp33, which is immunodominant in the context of both H-2Kb and H-2Db (29). Using transgenic mice with a TCR specific for the H-2Db-gp33 complex (p14 TCR), the potential for cross-reactive recognition of tissue-restricted self-peptides was examined. One of the self-epitopes identified was derived from dopamine β-mono-oxygenase (DBM) (18), an enzyme expressed in the adrenal medulla that is responsible for the conversion of dopamine to noradrenaline (30).

1 The abbreviations used are: TCR, T cell receptor; LCMV, lymphocytic choriomeningitis virus; MHC, major histocompatibility complex; CTL, cytotoxic T lymphocyte; DBM, dopamine β-mono-oxygenase; mβ2m, mouse β2-microglobulin.
Activated p14 TCR-transgenic T cells recognized target cells pulsed in vitro with either the mouse rDBM peptide (KALYDYAPI) or the rat homologue rDBM (KALYNAPI) (18). Interestingly, after in vivo activation of the transgenic T cell population with recombinant vaccinia virus expressing gp33 (vacc-GP) through LCVM infection or administration of the gp33 peptide, CD8+ T cell infiltration and inflammation of the adrenal medulla were detected in conjunction with significantly increased levels of dopamine in the adrenal glands (18). Importantly, infection with the LCVM variant 8.7 (which contains a single pV3L mutation in the gp33 epitope) as well as with control vaccinia VSV virus did not result in any infiltration of the adrenal medulla or in alterations of dopamine levels. Furthermore, the DBM peptide, sharing limited amino acid identity with gp33, could positively select the virus-specific p14 TCR in fetal thymic culture but was unable to induce clonal deletion (20). The self-epitope was expressed in complex with H-2Db on thymic epithelial cell lines and thymocytes that were selected by H-2Db-rDBM proliferated vigorously in response to H-2Db-gp33 complexes. In summary, the data of Ohteki et al. (18) and Saibil et al. (20) demonstrate that the DBM peptide (rDBM, KALYDYAPI) is processed and presented by H-2Db, mimicking the LCVM-derived gp33 epitope in complex with the same MHC molecule in vivo, initiating autoreactive T cell infiltration within the adrenal medulla of the treated mice. A rat DBM peptide homologue (rDBM, KALYNAPI), which only differs in position 5, was also identified in the study by Ohteki et al. (18). The rDBM peptide displayed similar properties to mDBM, stimulating proliferation and cytotoxicity of p14 T cells specific for the H-2Db-gp33 complex and acting as a partial agonist/antagonist. However, the rDBM peptide binds with a higher affinity to H-2Db, likely due to the presence of an asparagine residue at anchor position 5 instead of an aspartate in murine DBM (18).

The main purpose of this study was to provide a structural basis for activation of cross-reactive CD8+ T cells by epitope mimicry in a well-established mouse model. We present the crystal structure of H-2Db in complex with the rat homologue of the self-derived DBM peptide and its comparison with the virus-derived gp33 peptide, CD8+ T cell infiltration and inflammation of the adrenal medulla were detected in conjunction with significantly increased levels of dopamine in the adrenal glands (18). Importantly, infection with the LCVM variant 8.7 (which contains a single pV3L mutation in the gp33 epitope) as well as with control vaccinia VSV virus did not result in any infiltration of the adrenal medulla or in alterations of dopamine levels. Furthermore, the DBM peptide, sharing limited amino acid identity with gp33, could positively select the virus-specific p14 TCR in fetal thymic culture but was unable to induce clonal deletion (20). The self-epitope was expressed in complex with H-2Db on thymic epithelial cell lines and thymocytes that were selected by H-2Db-rDBM proliferated vigorously in response to H-2Db-gp33 complexes. In summary, the data of Ohteki et al. (18) and Saibil et al. (20) demonstrate that the DBM peptide (rDBM, KALYDYAPI) is processed and presented by H-2Db, mimicking the LCVM-derived gp33 epitope in complex with the same MHC molecule in vivo, initiating autoreactive T cell infiltration within the adrenal medulla of the treated mice. A rat DBM peptide homologue (rDBM, KALYNAPI), which only differs in position 5, was also identified in the study by Ohteki et al. (18). The rDBM peptide displayed similar properties to mDBM, stimulating proliferation and cytotoxicity of p14 T cells specific for the H-2Db-gp33 complex and acting as a partial agonist/antagonist. However, the rDBM peptide binds with a higher affinity to H-2Db, likely due to the presence of an asparagine residue at anchor position 5 instead of an aspartate in murine DBM (18).

The main purpose of this study was to provide a structural basis for activation of cross-reactive CD8+ T cells by epitope mimicry in a well-established mouse model. We present the crystal structure of H-2Db in complex with the rat homologue of the self-derived DBM peptide and its comparison with the previously determined crystal structures of H-2Db in complex with the cross-reactive immunodominant LCVM-derived gp33 peptide (31, 32) and the non-cross-reactive gp33 (V3L escape variant) (32). Although sharing only 56% sequence identity, the rat DBM and the virus-derived gp33 peptides are presented in nearly identical manners by H-2Db, with a striking conservation of peptide conformation, especially in the central segment corresponding to residues p3–p7. The positioning of the side chains that may interact with the TCR is conserved. The conformations of some residues of the H-2Db heavy chain are changed to accommodate the differences between the viral and self-peptides. However, these changes do not infer significant differences on the surface areas of both MHC complexes that interact with the TCR. This study provides a structural basis for peptide molecular mimicry inducing CD8-dependent T cell cross-reactivity. The structures allow us to explain how a dopamine β-mono-oxygenase-derived peptide, sharing only a partial sequence similarity with the immunodominant LCVM-derived peptide gp33, permits the cross-reactivity of the gp33-specific TCR p14.

**EXPERIMENTAL PROCEDURES**

**Preparation and Crystallization of the H-2Db-rDBM-mDBm MHC Complex**—The rDBM peptide (KALYNAPI) was purchased from Research Genetics (Huntsville, AL). The refolding of the H-2Db-rDBM MHC complex was conducted as previously described (31). Crystals were obtained in hanging drops by vapor diffusion. Crystal screens (Hampton Research, Laguna Niguel, CA) were used to establish initial crystallization conditions, which were then refined in a finer grid. The best crystals for the H-2Db-rDBM-mDBm complex were obtained in 1.8 M ammonium sulfate, 0.1 M Tris-Cl, pH 9.0, at room temperature. Typically, 4 μl of a 3 mg/ml protein solution in 20 mM Tris-Cl, pH 7.0, were mixed at a 2:2:1 ratio with the crystallization reservoir solution and ethanol. The drops were allowed to equilibrate at room temperature.

**Data Collection and Processing**—X-ray data were collected under cryogenic conditions (T = 100 K) to 2.7 Å resolution at beam line 1711 in MaxLab, Lund, Sweden (λ = 1.0292 Å) using a MAR345 image plate. Crystals were soaked for a short time in a cryoprotectant solution containing 30% glycerol before data collection. The diffraction data were processed and scaled using the HKL program package (35). Data statistics are presented in Table I. The crystal structure was solved by molecular replacement using the AMoRe program (34). The crystal structure of H-2Db-gp33-mDBm (31) with the peptide omitted was used as a search model. Refinement of the model was performed using the CNS suite of programs (35), and 4% of the reflections (2232 reflections) were set aside for monitoring the refinement by Rfree (36). The refinement started with rigid body refinement, treating the α1α2 and α3 domains of H-2Db as rigid bodies (37). An easily interpretable electron density was observed in the peptide binding cleft, and the peptide KALYNAPI could be placed unambiguously in all four MHC complexes. The model was further refined by restrained and isotropic individual B-factor positional refinement. Tight restrained non-crystallographic symmetry was used throughout the refinement. 109 water molecules were added to the complex using the water-pick option in CNS (30) (in the F–F′ map), and the positioning of all water molecules was inspected manually in O. The final rounds of refinement were performed with the program Refmac (38). TLS refinement yielded final R and Rfree values of 21.7% and 26.1%, respectively. The stereochemistry of the models was analyzed using PROCHECK (39). Refinement statistics are provided in Table I. Atomic coordinates and structure factors for the H-2Db-rDBM-mDBm complex have been deposited in the Protein Data Bank under accession code 1ZH2.

**RESULTS AND DISCUSSION**

We have determined the structure of H-2Db in complex with rat DBM peptide (KALYNAPI), which binds with a higher affinity to H-2Db than the mouse DBM peptide (KALYDYAPI). However, both the mouse and the rat DBM peptides are recognized by T cells expressing the H-2Db/LCVM gp33-specific p14 TCR, and both act as partial agonists/antagonists. Likewise, we have previously demonstrated that in the context of H-2Db, the structural conformations of the two most common sequence variants of the gp33 peptide, gp33(C) (KAVYNFATC) and gp33(M) (KAVYNFATM), are nearly identical (31). We will therefore refer to the virus-derived peptide gp33(M) as gp33 in the analyses presented hereafter.

**Overall Structure**—As expected, the overall structure of H-2Db in complex with the β-mono-oxygenase-derived peptide rDBM is very similar to previously reported MHC crystal structures. The Rfree and Rfree values for the refined model of the H-2Db-rDBM MHC are 21.7% and 26.1%, respectively (Table I). The stereochemistry is as expected for a model at this resolution (2.7 Å). The final electron density map is of good quality, with a well-defined polypeptide chain. In particular, the electron density for all residues in contact with the rDBM peptide and for the peptide is clearly defined (Fig. 1). The average B-factor is less than the average B-factor of the heavy and light chains (Table I). The overall structures of the MHC molecules H-2Db in complex with rDBM (KALYNAPI) or gp33 (KAVYNFATM) (31) are very similar, with a root mean square deviation of 0.70 Å2 for the whole complexes and 0.36 Å2 for the residues corresponding to the α1 and α2 domains (residues 1–182).

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Despite Large Sequence Disparity, the Conformations of the Peptides rDBM and gp33 Are Similar When Bound to H-2Db—The peptide rDBM shares only 56% sequence identity with the virus-derived epitope gp33. In particular, only one H-2Db anchor position is conserved in both epitopes (asparagine at position 5). Four other positions are conserved: lysine at p1, alanine at p2, tyrosine at p4, and alanine at p7. Conversely, rDBM and gp33 differ at four positions: 1) a valine in gp33 is replaced by a leucine in rDBM at p3, the secondary anchor position differs; 2) a tyrosine in gp33 is substituted with a phenylalanine in rDBM at p6, one of the main TCR-interacting positions; and 3) there are changes from a threonine and a methionine in gp33 to a proline and an isoleucine in rDBM at p8 and p9, respectively, at the C-terminal ends of both peptides. Some of these differences (e.g. the pV3L and pF6Y substitutions) may seem minor. However, when taken in the context of known functional and structural results, the cross-reactivity of the H-2Db/LCMV gp33-specific p14 TCR with H-2Db/rDBM is surprising.

Upon CTL selection pressure, LCMV virus escape variants emerge that are based on single amino acid mutations in gp33. Clones displaying three of the most common single mutations (pV3L, pY4F, or pF6L) in the LCMV gp33 sequence escape gp33-specific CTLs in the context of H-2Db (40–43). The escape peptides (all with 88% identity to gp33) abolish either all recognition by the p14 TCR or act as partial agonists/antagonists (43, 44). The mutation of a valine to a leucine at position 3 of gp33 also allows LCMV strains to escape immune recognition without markedly altering the binding affinity of the modified peptide to H-2Db (32). The V3L mutation results in a decreased affinity of the p14 TCR by ~40-fold compared with the wild type H-2Db/gp33 complex (43). Thus, conservative substitutions impair the binding of the p14 TCR to H-2Db in complex with mutated escape variants and result in abolished TCR recognition.

We have recently performed comparative structural studies of H-2Db in complex with either wild-type gp33 or each of the three single-mutated escape epitopes pV3L, pY4F, and pF6L described above (32). These studies revealed that the conservative mutations either induce localized conformational changes of peptide residues that interact with the TCR, such as p4 in H-2Db/gp33 (pV3L) and p6 in H-2Db/gp33 (pF6L), or result in two very similar structures with barely any differences except the removal of a hydroxyl group at the TCR/pMHC interface, such as in H-2Db/gp33 (pY4F) (32). Thus, the conservative escape variants introduced subtle structural modifications that mostly affected peptide residues that interact with the p14 TCR in the context of H-2Db. One of these escape substitutions (pV3L) is present in rDBM, and despite the limited sequence identity between rDBM and gp33, the p14 TCR is still able to interact with H-2Db in complex with either of these two peptides.

Both the gp33 and rDBM peptides have the characteristic conformation for peptides bound to H-2Db in that they bulge from residues 6–8 (45), with p1K, p7A, and, in particular, p4Y and p6F for gp33 and p6Y for rDBM projecting up into the solvent toward the TCR (Figs. 1 and 2). The peptide backbones align well with each other from residue p1K to residue p6F, whereas the backbone of rDBM shifts from residue 7 to residue 9 by 0.8 Å toward the peptide binding cleft of H-2Db (Fig. 3). The change from a valine in gp33 to a leucine in rDBM thus does not introduce any changes in the N-terminal half of the peptides. In conclusion, comparison of the bound peptide structures in H-2Db reveals essentially identical main chain conformations from p1K to p5N and only slight divergence at p6. The two epitopes gp33 and rDBM are very similar in conformation.

Table 1: Data collection and refinement statistics

| Data collection and refinement statistics | H-2Db/mβ2m/rDBM |
|------------------------------------------|------------------|
| Data collection                          |                  |
| Resolution (Å)                           | 25.0–2.7         |
| Measured reflections                     | 2605835          |
| Unique reflections                       | 58538            |
| Completeness (%)                         |                  |
| All data                                 | 98.8             |
| Highest resolution shell                 | 93.6             |
| Rmerge (%)                               | 8.1              |
| Highest resolution shell                 | 50.2             |
| I/σ(I)                                   |                  |
| All data                                 | 14.4             |
| Highest resolution shell                 | 1.7              |
| Mosaicity (%)                            | 0.6              |
| R-values from Wilson plot (Å²)           | 66.2             |
| Refinement statistics                    |                  |
| Resolution (Å)                           | 20.0–2.7         |
| Unique reflections                       | 53240            |
| Rcryst (%)                               | 21.7 (30.7)      |
| Rfree (%)                                | 26.2 (36.4)      |
| No. of protein atoms                     | 12552            |
| No. of waters                            | 109              |
| Average B-factor (Å²)                    |                  |
| Protein                                  | 56.9             |
| Peptide                                  | 43.9             |
| Water                                    | 56.0             |
| r.m.s. deviation, angles (°)             | 1.5              |
| r.m.s. deviation, bonds (Å)              | 0.008            |
| r.m.s. B-factor (Å²)                     |                  |
| Bonded atoms                             | 8.5              |
| Non-bonded atoms                         | 15.3             |
| Ramachandran plot (%)                    |                  |
| Residues in most favored regions         | 89.7             |
| Residues in disallowed regions           | 0                |

* Values in parentheses are calculated for the resolution interval 2.75–2.7.
* B-factors before TLS refinement.
* r.m.s., root mean square.

Fig. 1. Electron density map for the dopamine β-mono-oxygenase-derived rDBM and the LCMV-derived gp33 peptides. Annealed omit 2Fo – Fc electron density maps of gp33 (top panel) and rDBM (bottom panel) when bound to H-2Db contoured at 1.0σ. The peptides are depicted with their N termini at the left and their C termini at the right, and the anchor positions are indicated by vertical arrows.
Structural Basis for CD8 T Cell Cross-reactivity

Fig. 2. The peptides rDBM and gp33 bound to the cleft of the MHC class I H-2Db molecule. The peptides are represented as stick models, whereas the MHC class I molecule is represented by its surface (as viewed from above). The pockets permitting binding of different sections of the two peptides are indicated. The positions of H-2Db residues important for the binding of rDBM and gp33, as well as a few other residues, are indicated. Negatively charged regions of the surface are shown in red, and positively charged regions are shown in blue, with a scale from −15 to +15 kiloteslas. Peptide residue labels begin with p. This figure was created using the Grasp program (68).

at the three TCR-interacting positions p1, p4, and p6.

The conserved conformation of the peptides between H-2Db-rDBM and H-2Db-gp33 is not a general feature of MHC class I structures, as evident from previous structural studies of MHC class I molecules in complex with different peptides (46–49). The groups of Ian Wilson and the late Don Wiley (46, 48, 49) solved the structures of H-2Kb, RT1-Aa, and HLA-A2 in complex with different peptides, clearly demonstrating that the epitopes were tethered at each terminus of the peptide-binding cleft. Whereas residues p1, p2, and the C-terminal anchor side chain bound similarly in all cases, the main chain and side chain conformations of each peptide are strikingly different in the center of the binding site (residues p3–p7), providing an antigenic identity for each peptide-MHC complex (46, 48, 49).

Infection of p14 transgenic mice with the LCMV variant 8.7 (which contains a single pV3L mutation in the gp33 epitope) does not result in any infiltration of the adrenal medulla or in alterations of dopamine levels (18). Thus, the information retrieved from the crystal structure of the gp33 (V3L)-altered peptide escape variant in complex with H-2Db (32) is of special interest to the present study. The structure of H-2Db-gp33 (V3L) revealed that the introduction of the larger leucine side chain resulted in a small movement of the peptide toward the α1 domain of the H-2Db peptide-binding cleft. The observed Cα shifts occurred in residues p2A, p3L, and p4Y (Fig. 3). The positioning of the side chain of the latter residue, a potential main TCR contact, was also affected, and the side chain moved upwards toward the TCR. In summary, the small structural shifts observed in the crystal structure of the V3L viral escape variant are sufficient to abolish recognition by the p14 TCR, despite the fact that it shares 88% identity with gp33 and has identical side chains at TCR-contacting residues p1K, p4Y, and p6F.

The striking conformational similarity of gp33 and rDBM, especially between residues p3 and p6, thus suggests the importance of a shared structural antigenic identity for molecular mimicry, a concept that has received support from functional and structural studies of TCR/MHC class II systems. Lang et al. (50) recently demonstrated that the CD4+ T cell clone Hy.2E11, isolated from a patient suffering from multiple sclerosis, recognizes two separate peptides with limited conservation of crucial residues in the context of two different MHC class II molecules (50). Hy.2E11 binds to both the DRB1*1501-restricted self-peptide MBP 85–99 derived from myelin basic protein and a DRB5*0101-restricted Epstein-Barr virus-derived peptide (50, 51). As observed in the two structures presented within this study, the self-MPB and the virus-derived peptides aligned well from residue p(-1) to residue p5. The positioning and identity of four residues (p(-1)I, p2H, p3F, and p5K) of importance for the interaction with the TCR were conserved. TCR cross-reactivity with MHC class I-peptide complexes seems to follow the same basis as that for MHC class II because the positioning (if not the identity) of the residues of importance for interaction with TCR is conserved (Fig. 3).

The case of residue p6 (phenylalanine in gp33 and tyrosine in rDBM), formerly demonstrated as being important for binding and recognition of H-2Db-gp33/m4β4 by the CTLs, is interesting (43, 52). The potential sterical clash due to the presence of an additional hydroxyl group that could be created at the TCR-pMHC interface in the case of the rDBM peptide is removed because the backbone of the peptide shifts slightly toward its binding groove, away from the TCR (Fig. 3). This shift is possible due to the movement of the main anchor position p9I deeper within the F-pocket, allowing for a better fit of this hydrophobic cavity. The presence of a proline at position 8 does not seem to affect the overall conformation of rDBM in any major way. Superposition of the α1α2 domains corresponding to heavy chain residues 1–182 (which form the peptide binding cleft) reveals that the two H-2Db heavy chain Cα-backbones align well with each other in the regions corresponding to the first half of the bound peptides. However, although all of the heavy chain residues assume similar conformations, the C-terminal part of the peptide-binding groove is wider in H-2Db than in H-2Db-gp33, most probably in order to better accommodate the DBM peptide that binds deeper in the H-2Db cleft. Such limited displacements localized at the vicinity of the peptide C terminus have previously been reported in other structural studies of cross-reactive TCR/pMHC molecules (50, 53).

In conclusion, the conformation of all peptide residues that are likely to interact with the TCR is conserved between gp33 and rDBM when presented by H-2Db. Both peptides display similar antigenic identity between residues p3 and p7. Furthermore, the conformations of the two peptides are such that small but important differences, such as the presence or absence of a hydroxyl group at the TCR-pMHC interface, are compensated for by relative movements of the peptide backbone so that no too close contacts/sterical clashes can occur at the interface between the p14 TCR and the H-2Db-rDBM complex. The occurrence of such small disturbances at the TCR-peptide interface has been demonstrated to be sufficient for agonistic versus antagonistic TCR signaling (54, 55).
The Surfaces of H-2Db in Complex with Either rDBM or gp33 Are Nearly Identical: A Potential Structural Basis for Molecular Mimicry and Induction of Autoreactivity—Molecular mimicry may be described in structural terms as a similarity in charge distribution as well as overall shape for an interaction surface (50). Although the conformations of some side chains of the H-2Db heavy chain are slightly altered to encompass the structural modifications introduced by the differences between the two peptides, it should be noted that the movements of these side chains are very subtle and that most are not perceived at the pMHC surface that interacts with the TCR (Figs. 2 and 4). The conformation of all the H-2Db residues that potentially contribute to the TCR binding surface is essentially conserved between the structures of H-2Db/rDBM and H-2Db/gp33.

All of the co-crystal structures of TCR and MHC-peptide (pMHC) complexes determined to date (56), with one exception (57), have demonstrated a similar docking orientation of the TCR on the pMHC. TCRs recognize pMHC with a roughly diagonal orientation, but none of these crystal structures share sets of common contacts (56). The variable domains of the TCR α and β chains (CDR1–3) determine the specificity of T cell recognition. The CDR 3 regions have a primary role in readout of the bound peptide antigen, whereas CDR 1 and 2 regions tend to recognize conserved features of the MHC helices. It also appears from these studies that conformational changes in the TCR CDR loops are the primary mechanism for enhancing TCR cross-reactivity and that structural complementarity in the TCR-pMHC interface plays an important role in the half-life and functional consequences of TCR-pMHC interactions. Although we have not yet succeeded to date in co-crystallizing the p14 TCR with either of the MHC complexes, we can conclude from the two H-2Db structures presented within this study that 1) the strict conformational juxtaposition of the p3–p7 segments of the gp33 and rDBM peptides provides a structural basis for the ability of the p14 TCR to cross-react, 2) no structural rearrangement of the TCR would be needed in p14 in order to recognize both MHC structures, and 3) the structural complementarity between any of these two MHC complexes and the p14 TCR is most probably kept at a very similar level.

Our results extend previous studies of MHC class II complexes demonstrating that the similarity of the antigenic surfaces rather than sequence homology dictates T cell epitope cross-reactivity and molecular mimicry (8, 50). Some specific TCRs may cross-react with two MHC class II complexes despite restricted allelic differences. For example, the crystal struc-
tures of the human TCR HA1.7 in complex with either DRα*0101 or DRB1*0401 MHC class II molecules are very similar, despite local conformational changes of the hemagglutinin peptide induced by the MHC allelic differences (58, 59). Most of the allelic differences are located deep in the peptide binding cleft and are not directly accessible to the TCR. However, these differences induce very small structural conformational changes in the side chains of two peptide residues at p5 and p6. These minimal changes do not introduce major modifications at the surface of both MHC class II molecules and are tolerated by the HA1.7 TCR but are sensed by other TCRs (59).

CONCLUSIONS

Although the contribution of nonspecific inflammatory mechanisms has received experimental support in several different animal models of autoimmune disease (60, 61), there is also considerable evidence for the concept of epitope mimicry in stimulating autoreactive T cells (13, 19, 50, 62–66). Two mutually non-exclusive mechanisms are considered important in T cell epitope mimicry (62). The first is based on the sharing of CD4-related molecular mimicry (62). The first is based on the sharing of critical residues between the foreign and the self-peptide required for T cell activation. The second is based on the overall structural similarity of the MHC(s) heavy chain in complex with different peptides. Here we provide a structural example for peptide molecular mimicry-induced CD8+ T cell cross-reactivity. The findings presented here are well in line with previous structural studies of CD4-related molecular mimicry (50) and suggest that further structural studies of T cell cross-reactivity and epitope mimicry will be crucial for understanding the activation of autoreactive T cells and subsequent development of autoimmune disease.

Addendum—It has come to our attention during the production of this work that the research group of Dr. B. Uchanska-Ziegler has demonstrated an allele-dependent molecular mimicry between viral and self-peptide (67). Using x-ray crystallography, Fiorillo et al. (67) demonstrated that the Epstein-Barr virus peptide pLMP2 is presented in two diametrically opposed manners when binding to either HLA-B*2705 or HLA-B*2709. Furthermore, an extensive structural similarity between pLMP2 and the self-peptide pVIPR, derived from viral antigens, is evident. These minimal changes do not introduce major modifications into the pT4 T cell cross-reactivity with different peptides. Here we provide a structural example of peptide molecular mimicry-induced CD8+ T cell cross-reactivity (62).

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A Structural Basis for CD8\(^{+}\) T Cell-dependent Recognition of Non-homologous Peptide Ligands: IMPLICATIONS FOR MOLECULAR MIMICRY IN AUTOREACTIVITY

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*J. Biol. Chem.* 2005, 280:27069-27075.
doi: 10.1074/jbc.M500927200 originally published online April 21, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M500927200

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