Alloreactivity between Disparate Cognate and Allogeneic pMHC-I Complexes Is the Result of Highly Focused, Peptide-dependent Structural Mimicry

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Our understanding of the molecular mechanisms of T cell alloreactivity remains limited by the lack of systems for which both the T cell receptor allo- and cognate ligand are known. Here we provide evidence that a single alloreactive T cell receptor interacts with analogous structural regions of its cognate ligand, HLA-B*0801FLRGRAYGL, as its allogeneic ligand, HLA-B*3501KPIVLHGTY. The crystal structures of the binary peptide-major histocompatibility complexes show marked differences in the conformation of the heavy chains as well as the bound peptides. Nevertheless, both epitopes possess a prominent solvent-exposed aromatic residue at position 7 flanked by a small glycine at position 8 of the peptide determinant. Moreover, regions of close structural homology between the heavy chains of HLA B8 and HLA B35 coincided with regions that have previously been implicated in “hot spots” of T cell receptor recognition. The avidity of this human T cell receptor was also comparable for the allo- and cognate ligand, consistent with “non-self” or allogeneic MHC molecules, with some studies estimating alloreactive T cell frequencies of up to 10% (2). Direct T cell alloreactivity contributes significantly to complications associated with organ transplantation and is a long-standing paradox in cellular immunity. In a clinical context, T cell allore cognition manifests as graft versus host disease in bone marrow transplantation or graft rejection in solid organ transplantation. Despite advances made using immunosuppressive agents, both graft versus host disease and organ rejection are still associated with a high degree of morbidity and mortality. Thus, a better understanding of the mechanism of T cell alloreactivity is needed to further advance post transplant therapies.

Various attempts to explain the molecular interactions that lead to TCR alloreactivity have resulted in two main schools of thought. One model proposes that the alloreactive TCR directly recognizes polymorphisms in the allo-MHC molecules independent of the bound peptide, giving rise to a high “antigen density” (3). Alternatively, if the TCR perceives the similarities of the foreign MHC molecule and focuses on one or more of the diverse peptides that appear foreign when presented by allogeneic MHC molecules, this constitutes a “determinant frequency” alloresponse. In this second model, it is argued that the many thousands of “foreign” peptides would create at least one determinant for cross-reactive recognition by the TCR (4). The latter model includes T cell responses to peptides that differ in sequence as well as responses to the same peptide adopting different conformations when bound by the allo and self MHC molecules. Examples of allore sponses demonstrating a range of peptide

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9 The on-line version of this article (available at http://www.jbc.org) contains supplemental Fig. 1.

The atomic coordinates and structure factors (code 2H6P) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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7 The abbreviations used are: TCR, T cell receptor; MHC, major histocompatibility complex; EBV, Epstein-Barr virus; CTL, cytotoxic T lymphocytes; CDR, complementarity-determining region; r.m.s.d., root mean square deviation; FACS, fluorescence-activated cell sorter; HLA, human leukocyte antigen; PE, phycoerythrin.
dependence have been documented from T cells recognizing alloantigens in a more “peptide-independent” manner (5–7) to alloresponses that are peptide-dependent (8–14). The spectrum of peptide-dependent (and specific) alloresponses is considered to vary according to the level of similarities between the self-MHC and allo-MHC molecules, with increased peptide-specific responses observed with increased similarities (15).

Understanding the molecular and structural basis of T cell alloreactivity has been limited by the lack of available systems for which the sequences of the TCR, syngeneic ligand, and allogenic ligands are all known. The crystal structures have been determined for the 2C-H2-Kbm3/H2-Kb system (8), demonstrating a shared docking mode of the 2C TCR on the alloligand and the syngeneic ligand. In this system, the sequence of the alloligand peptide is identical to the cognate peptide, and the H2-Kbm3 and H2-Kb molecules differ by only two amino acids. Another study compared the crystal structures of BM3.3 TCR in complex with H-2Kb presenting either the VSV8 octamer or the naturally processed peptide, pBM1 (16). These systems have given some insight into TCR cross-reactivity but have limitations as they have either identical peptides or identical MHC molecules. As such, they may not adequately explain allorecognition involving very disparate MHC class I allotypes and alloligand peptides of differing amino acid sequence(s).

We chose to study an alloreactive human T cell clone, termed JL9, that recognizes the peptide FLRGRAYGL (referred to as FLR), an immunodominant epitope derived from the latent Epstein-Barr virus (EBV) nuclear antigen 3A (EBNA3A), bound to HLA-B*0801 (17, 18). The JL9 cytotoxic T lymphocyte (CTL) clone was alloreactive against HLA-B*3501, as demonstrated by lysis of HLA-B*3501-expressing target cells in the presence of a peptide derived from human cytochrome P450, KPIVVLHGY (referred to as KPI) (18). In this system there is little similarity between the cognate and allopeptide, and HLA-B*3501 differs from HLA-B*0801 by 19 amino acids. The basis for the JL9 TCR alloreactivity was examined by avidity measurements, peptide substitution studies, and x-ray crystallography. Our data are consistent with T cell allorecognition being dependent on highly focused structural mimicry between the disparate cognate and allogenic allotypes.

**EXPERIMENTAL PROCEDURES**

**Flow Cytometric Analysis**—T cell clones or peripheral blood mononuclear cells were incubated for 30 min at 4 °C with a PE-labeled HLA-B*0801 FLR pentamer and/or an antigen-presenting cell-labeled HLA-B*3501 KPI pentamer (ProImmune). Cells were then washed twice in FACS buffer (1% fetal calf serum in PBS) and labeled for 30 min at 4 °C with Tri-color-labeled anti-human CD8 monoclonal antibody (Caltag Laboratories). The cells were then washed with FACS buffer, fixed with Cytofix (BD Biosciences), and analyzed on a FACSCanto using FACS.Divia software (BD Biosciences). For the pentamer dilution analysis, T cell clones (2 × 10^6 cells/ml) were incubated with increasing dilutions of pentamer (1:100–1:51200) in 100 μl of FACS buffer for 30 min at 4 °C. The cells were then washed 3 times with FACS buffer, fixed, and analyzed as described above.

**T Cell Cytotoxicity Assays**—CTL clones were tested in duplicate in the standard 5-h chromium release assay for cytotoxicity against ^51^Cr-labeled target cells that had been treated for 1 h with various concentrations of peptide. The target cells were HLA-B*0801^+^ or HLA-B*3501^+^ phytohemagglutinin blasts that were raised by stimulating peripheral blood mononuclear cells with phytohemagglutinin followed by propagation in interleukin-2-containing medium for up to 8 weeks. Target cells were added to effector cells at a ratio of 1:1. Percent specific lysis was calculated, and the peptide concentration required for half-maximum lysis was determined from dose-response curves. Peptides were synthesized by Mimotopes. A β scintillation counter (Topcount Microplate; Packard Instrument Co.) was used to measure ^51^Cr levels in assay supernatant samples. The mean spontaneous lysis for targets in culture medium was always <20%, and variation about the mean specific lysis was <10%.

**Expression, Purification, and Crystallization of HLA B*3501**—Soluble HLA-B*3501 molecules (residues 1–276) and full-length β2-microglobulin (residues 1–99) were expressed, refolded with the KPIVVLHGY peptide, purified, and concentrated to 10 mg/ml as previously described (19). The HLA-B*3501 KPI crystals were obtained by the hanging drop vapor diffusion method. Block-shaped crystals grew within 10 days in conditions containing 0.2 M ammonium acetate and 18% w/v polyethylene glycol 3350 (100 mm cacodylate, pH 7.6) at 4 °C.

**X-ray Data Collection and Structure Determination**—Crystals were soaked in reservoir solution containing increasing increments of glycerol as a cryoprotectant (5, 10, 15, and 20%) and then flash-frozen before data collection. Data were collected on an in-house radiation source (a Rikagu RU-3HBR rotating anode generator) and processed and scaled using the HKL suite (20). The HLA-B*3501 KPI structure was refined from a HLA-B*3501 structure that was previously determined in our laboratory. The model was manually built using the program CNS (21) and improved through multiple rounds of refinement using the CNS suite (22). The progress of refinement was monitored by the Rfactor and the Rfree values. Rigid body refinement and simulated annealing were used in the first instance, and later rounds of energy minimization and B-individual refinement were used to improve the quality of the model. After this, more rounds of refinement were implemented using the REFMAC (23) program and subsequent model building using WinCoot (24). Water molecules were included if they had a B-factor less than 60 Å^2^, appeared in Fobs − Fcalc maps contoured at 3.5 σ and were within hydrogen-bonding distance to chemically reasonable groups. See Table 1 for the final refinement and model statistics. The structure has been deposited in the Protein Data Bank under code 2H6P.

**RESULTS**

Cross-reactivity of an HLA-B8/EBV-reactive T Cell Clone with the Alloantigen HLA-B*3501 KPIVVLHGY—HLA-B*0801-restricted T cell clones specific for the EBV epitope FLRGRAYGL isolated from unrelated HLA-B8^+, B44^+ individuals have pre-

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viously been shown to alloreact with HLA-B*3501-expressing cells (18). To investigate the mechanism of T cell alloreactivity, the binding specificities of a representative EBV/allo-cross-reactive T cell clone (called JL9) were first investigated. MHC pentamers of the cognate viral ligand HLA-B*0801FLR and the alloligand HLA-B*3501KI were used to co-stain the CD8+ JL9 T cell clone (Fig. 1A). The JL9 T cells recognized the alloligand and the cognate viral ligand with the same level of specificity because both pentamers were able to specifically react with the JL9 clone simultaneously and with very similar levels of fluorescence intensity. As controls, the same pentamers were used to stain the FLR-specific, HLA-B*0801-restricted CTL clone LC13, which is not alloreactive with HLA-B*3501 (17) (Fig. 1B), and the ELS4 CTL clone, which is specific for another EBV epitope and is not alloreactive (25) (Fig. 1C).

To determine whether T cells with this dual specificity can be detected ex vivo, peripheral blood mononuclear cells were isolated from two EBV-seropositive, HLA-B*0801+, B*4402+ donors and tested for their ability to bind HLA-B*0801FLR and HLA-B*3501KI. A small but detectable population of HLA-B*3501KI alloligand T cells (0.2% of CD8+ cells) was observed directly ex vivo in both donors (supplemental Fig. 1). Donors with the HLA-B8+, B44+ phenotype typically have a low frequency of HLA-B*0801FLR specific memory T cells ex vivo (26).

T Cell Avidity and Affinity—Fluorescently labeled, soluble MHC multimers can detect differences in avidities of MHC-specific T cells using flow cytometry (27–30). When MHC multimers are used as a limiting reagent, only high avidity T cells bind the multimers, while using the multimers at saturating levels identifies both low and high avidity T cells. To determine whether there is a difference in T cell avidity for an alloligand compared with a syngeneic ligand, the alloreactive T cell clone JL9 was stained with limiting dilutions of pentamers of the alloligand HLA-B*3501KI and the syngeneic ligand HLA-B*0801FLR. The JL9 T cell clone was stained over a range of pentamer dilutions from 1:100 to 1:51,200 (Fig. 2). The staining profile for the two pentamers followed the same trend at each dilution, with the limiting point at which pentamer binding ceased being observed at the 1:6400 dilution for both the allo pentamer and the cognate pentamer. Consistent with data shown in Fig. 1B, the control LC13 CTL clone stained only with the HLA-B*0801FLR pentamer. These results reveal that the JL9 TCR shows no significant difference in avidity for the allo ligand compared with the cognate ligand.

Fine Peptide Specificity Analysis of the Alloreactive TCR—To investigate how the single TCR expressed by the JL9 CTL clone binds with similar avidity to these two disparate target ligands, we conducted a fine peptide specificity analysis using altered peptide ligands presented in the context of either HLA-B*0801 or HLA-B*3501. An earlier study that examined the fine specificity of this CTL clonotype by testing for recognition of analogues of the FLR peptide at a single peptide concentration identified positions 6–8 as the likely TCR contact residues (18). The JL9 CTLs were, therefore, tested for their ability to tolerate single amino acid substitutions at positions 6, 7, and 8 within the FLR and KPI peptides. A total of 57 single amino acid-substituted analogues of both the FLR and KPI peptides were tested for CTL recognition using peptide dose-response cytotoxicity assays with phytohemagglutinin blast target cells expressing either HLA-B*0801 (Fig. 3A) or HLA-B*3501 (Fig.
Although the total number of peptide amino acid substitutions that prevented lysis by JL9 was greater for the HLA-B*3501KPI target ligand than for the HLA-B*0801FLR complex, the total number of peptide amino acid substitutions that led to a 10-fold loss of lytic activity was very similar for the two complexes. The JL9 CTL clone was more sensitive to substitutions at position 6 in the HLA-B*3501KPI complex in comparison to the HLA-B*0801FLR complex, which may reflect differential roles for position 6 in MHC binding, since the Leu at this position points in toward the MHC cleft in the HLA-B*3501KPI complex, whereas the Ala at position 6 is a solvent-accessible residue in the HLA-B*0801FLR complex (see below). The small Gly residue at position 8 was critical for recognition of both complexes. Furthermore, the JL9 CTL clone displayed a preference for an aromatic side chain at position 7 within each complex, indicating that this is an important TCR contact residue in both complexes.

Crystal Structure of the HLA-B*3501KPIVVLHGY Complex—
The crystal structure of HLA-B*3501KPI was determined to 1.9 Å and to an R factor of 21% and an R free of 23.1% (Table 1). The electron density of the bound peptide KPIVVLHGY and the residues contacting it in HLA-B*3501 was unambiguous, providing a clear view of the allogeneic pMHC landscape (Fig. 4). The HLA heavy chain (residues 1–276) and β2-microglobulin (residues 1–99) adopted the expected MHC class I and immunoglobulin-like folds, respectively (31). The peptide, which bound in an extended conformation within the antigen-binding cleft, was observed to exhibit limited flexibility (Fig. 4), with all residues possessing similar B-factors (ranging from 29 to 34 Å²). Consequently, the KPI peptide participates in numerous van der Waals interactions, 12 direct H-bonds, and 4 water-mediated H-bonds with the heavy chain (Table 2). The anchor residues for peptide binding to HLA-B*3501 are located at positions 2 and 9. The P2-Pro and P9-Tyr were observed to make the standard anchor contacts in the B- and F-pockets, respectively, within HLA-B*3501 (25, 32–34). The P3 and P6 positions also display limited solvent accessibility, with the hydrophobic P3-Ile and P6-Leu pointing into, and making numerous contacts with the antigen binding cleft. The solvent-exposed residues of the peptide are P1-Lys, P4-Val, P5-Val, and P7-His, with solvent exposure values of 49, 65, 42, and 91 Å², respectively; accordingly the most prominent P7-His residue appears to be an important contact point for the JL9 TCR.

Comparison of the Cognate and Allogeneic Complexes—
Given that the JL9 T cell clone lysed both HLA-B*3501KPI and B*0801FLR, expressing cells with comparable efficiency and binds to pentamers of both ligands with equal avidity, the overall structures of these two pMHC complexes were compared to better understand the structural basis of allorecognition. HLA-B*3501 and HLA-B*0801 differ by 19 amino acids, with most of the differences residing within the peptide binding cleft and not directly accessible to the JL9 TCR (Fig. 5). Of the polymorphic residues, two positions (97 and 116) participate in peptide binding (Fig. 5), and two positions (131 and 163) map to the anti-
genic surface (Fig. 6, A and B). Three of the polymorphic positions, 156 (Asp→Leu), 163 (Thr→Leu), and 194 (Ile→Val) superpose poorly, with root mean square deviation (r.m.s.d.) for these positions of 1.23, 1.04, and 1.38 Å, respectively. Overall, the antigen binding clefts of HLA-B*3501 and HLA-B*0801 superposed relatively poorly, with an overall r.m.s.d. of 0.85 Å. Considering that the JL9 TCR interacts with both of these peptide concentration required for half-maximal lysis by the JL9 CTL clone is shown for the various peptide analogs. The amino acid substitutions are shown on the vertical axis, and the log molar concentration of peptide analog is shown on the horizontal axis. The effector/target ratio employed for the replacement assay was 1:1.

TABLE 1
Crystallographic statistics for HLA-B*3501KPIVVLHGY
Values in parentheses are for the highest resolution shell.

| Data collection | Value |
|-----------------|-------|
| Temperature     | 100 K |
| Space group     | P2_12_2 |
| Cell dimensions (Å) | 51.22, 82.01, 110.26 |
| Resolution (Å)  | 1.9   |
| Total number of observations | 180277 |
| Number of unique observations | 36,699 |
| Multiplicity    | 4.91  |
| Data completeness (%) | 98.1 (83.6) |
| No. data >2σI (%) | 81.8 (41.3) |
| Rmerge (%)      | 20.7 (2.15) |
| Rfree (%)       | 9.1 (54.8) |

| Model refinement | Value |
|-----------------|-------|
| Non-hydrogen atoms | Protein: 3,156, Water: 401 |
|                | Rmerge (%) | 21.0 |
|                | Rfree (%)   | 23.9 |
|                | r.m.s.d. from ideality |
|                | Bond lengths (Å) | 0.006 |
|                | Bond angles (°) | 1.27 |
|                | Dihedrals (°) | T: 24.99, Improvers (°) | 0.807 |
|                | Ramachandran plot | Most favored region: 91.9, Allowed region (%): 8.1 |
|                | B-factors (Å²) | Average main chain: 33.55, Average side chain and water molecules: 36.07 |
|                | r.m.s.d.-bonded B-factors | 1.24 |

FIGURE 4. Structure of the allogeneic stimulating peptide, KPI, complexed to the MHC. The cytochrome P450 derived peptide, KPIVVLHGY (aqua), sitting in the peptide binding cleft of the MHC, HLA-B*3501 (green). The surrounding final 2Fo-Fc electron density for the peptide is shown in mesh format. This figure shows the positions of key anchor residues (Pro² and Tyr⁵) and possible TCR contact residues (Val⁴, Val⁵, and His⁷).

peptide concentration required for half-maximal lysis by the JL9 CTL clone is shown for the various peptide analogs. The amino acid substitutions are shown on the vertical axis, and the log molar concentration of peptide analog is shown on the horizontal axis. The effector/target ratio employed for the replacement assay was 1:1.

FIGURE 3. Peptide repertoire for the JL9 CTL. Amino acid substitution experiments depict how well the JL9 CTL clone tolerates amino acid changes at positions 6, 7, and 8 within FLR (A) versus KPI (B). The log
the interaction, with an r.m.s.d. of 0.18 and 0.20 Å for each Cα region of the two peptides adopt the most similar conformations (Fig. 6).

Interestingly, the JL9 TCR clone displayed a stringent requirement for a glycine at P8 for cytolytic activity, which is important for the JL9 TCR to form main chain interactions with P8 and/or that a small residue is required to allow optimal engagement of the bulky residue at P7 by the JL9 TCR. These requirements at P7 and P8 for recognition by the JL9 TCR are comparable with those observed for recognition of HLA-B*0801FLR by an immunodominant TCR called LC13 (35). Like the JL9 clone, the LC13 CTL clone is most sensitive to substitutions at the C-terminal end of the FLR peptide, as demonstrated by single amino acid substitution experiments (18, 35). Correspondingly, the peptide substitution experiment for the KPI peptide in the current study suggests that the JL9 TCR has a similar C-terminal based footprint on the alloligand HLA-B*3501 (Fig. 6C).

**DISCUSSION**

In unrelated HLA-B*0801+ individuals, the CD8+ T cell response toward the EBV latent epitope FLRGRAYGL is characterized by use of an immunodominant TCR, termed LC13 (36). Single amino acid substitution experiments of the FLR peptide bound to HLA-B*0801 revealed that the LC13 CTL clone was very sensitive to substitutions toward the C terminus of the peptide, specifically the highly exposed P7-Tyr, and the two small residues that flank this position (35). The crystal structure of the LC13-HLA-B*0801FLR complex provided a basis for this specificity whereby the P7-Tyr was enveloped within a centrally located pocket of the LC13 TCR, with the P6-Ala and P8-Gly forming main chain hydrogen bonds to the LC13 TCR (35, 37). To optimally engage the P7-Tyr of the FLR peptide, the LC13 TCR was observed to dock diamonically and adopt a C-terminal focused footprint onto HLA-B*0801FLR. Interestingly, the LC13 TCR is alloreactive against HLA-B4402, and in HLA-B8* B44+ heterozygous individuals the LC13 TCR is deleted from the repertoire, presumably as a consequence of negative selection during thymic development (26, 38). In these individuals, T cells expressing alternative TCRs are selected to interact with HLA-B*0801FLR, including the JL9 CTL clone. The JL9 T cell clone is another example of an HLA-B*0801FLR-specific clonotype that uses identical TCR chains.

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**TABLE 2: Interactions between KPIVVLHG and HLA-B*3501**

| Peptide residue | HLA-B35 residues | Interaction |
|-----------------|------------------|-------------|
| Lys1 | Tyr7, Arg62, Tyr159, Trp147 | Van der Waals |
| Lys2 | Tyr7, Ser7, Asn69, Ala149 | H-bond |
| Lys3 | Leu65, Asn155, Met149 | Van der Waals |
| Lys4 | Leu7, Ser7, Asn69, Ala149 | H-bond |

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**FIGURE 5. Polymorphisms between HLA-B*3501 and HLA-B*0801.** This figure shows a top view of the MHC molecule, highlighting the polymorphic residues between HLA-B*3501 and HLA-B*0801 as would be seen by a T cell receptor. The HLA-B*0801 residue is listed first, followed by the residue number and the corresponding HLA-B*3501 residue. The polymorphic residues that are involved with binding of the KPI peptide to HLA-B*3501 are colored in red; all other polymorphic residues are colored in yellow.
Structural Insights into T Cell Allorecognition

FIGURE 6. Highly focused molecular mimicry between the allo- and cognate ligands explains JL9 cross-reactivity. Surface representation of (self-)HLA-B*0801FLR (A) and allo-HLA-B*3501KPI (B). The surface of HLA-B*0801FLR and HLA-B*3501KPI differ, especially around the peptides and their binding clefts. The polymorphic residues are colored in yellow, the FLRGRAYGL peptide is colored in pink, and the KPIVVLHGY peptide is colored in aqua. For the polymorphic residues, the HLA-B*0801 residue is listed first followed by the peptide number and then the corresponding HLA-B*3501 residue. The energetic hot spot residues for recognition of HLA-B*0801FLR by the LC13 TCR are also shown (yellow). C, overlay of the α1 and α2 helices of HLA-B*0801 (blue) and HLA-B*3501 (green). The FLR peptide is colored in pink, and the KPI peptide is colored in aqua. The CDR loops of the LC13 TCR are shown in orange. The MHC residue positions known to be important for LC13 recognition of HLA-B*0801FLR are highlighted in yellow. In particular, the three residues of the restriction triad at positions 65, 69, and 155 appear to be conserved structurally.

(TRA1-2*01, TRAJ36*01, and TRBV4-3*01, TRBJ2-5*01, TRBD2*02) that are found in unrelated individuals. The JL9 TCR is also known to be alloreactive against a peptide derived from cytochrome P450, KPIVVLHGY, in the context of HLA-B*3501 (18).

In this report we have determined the crystal structure of the allogeneic pMHC complex HLA-B*3501KPI and compared it to the previously determined structure of the JL9 cognate ligand HLA-B*0801FLR (39). A comparison of these crystal structures combined with functional data for the alloreactive T cell clone JL9 has provided insight into the structural mechanism of the alloreactive T cell response. In the flow cytometry experiments, both the HLA-B*3501KPI and HLA-B*0801FLR pentamers positively stained the JL9 T cell clone, demonstrating the structural plasticity of the JL9 TCR. Moreover, the JL9 clone reacted with similar avidity to the HLA-B*3501KPI and the HLA-B*0801FLR pentamers. Early studies suggested that TCRs had a much higher affinity for alloglands compared with syngeneic ligands (40, 41). Although additional studies have demonstrated a similarity in the functional avidity of a TCR for an allogeneic and syngeneic ligand (42), the small number of cases for which both an allogeneic and cognate ligand are known for a particular TCR limits the availability of affinity data. Interestingly, the affinity of the well studied alloreactive 2C TCR differs according to the type of allogeneic ligand it recognizes (43). Our present study represents the first report of a TCR reacting with comparable avidities for an allogland and a cognate ligand.

The peptide substitution data revealed that the JL9 TCR was exquisitely sensitive to amino acid replacement at positions 7 and 8 of the cognate peptide, analogous to that exhibited by the LC13 TCR (35), suggesting that the JL9 TCR also forms a C-terminal footprint on HLA-B*0801. Moreover, the JL9 TCR was sensitive to substitutions at positions 7 and 8 of the allopeptide (P7-His, P8-Gly), which is consistent with the requirement for an aromatic residue at position 7, and a flanking glycine residue. This observation is also consistent with the JL9 TCR having a C-terminal biased footprint on the alloligand HLA-B*3501KPI that is similar to the cognate HLA-B*0801FLR complex. Although the allogeneic and cognate peptide differed markedly in sequence and in the overall conformation within their respective antigen binding clefts, they were observed to possess structural similarity and adopt a homologous conformation at positions 7 and 8. The localized bulge at P7 in the KPI peptide (P7-His) and the FLR peptide (P7-Tyr) provides a conserved prominent feature that is likely to form similar interactions with the JL9 TCR, possibly in an analogous mode observed for LC13 binding to the FLR peptide (35).

As with most TCR/pMHC complexes, the JL9 TCR will also make extensive contacts with the MHC heavy chain. In this regard, it was of interest to observe that the antigen-binding cleft of HLA-B*0801 and HLA-B*3501 did not overlay closely. This was somewhat surprising, given that patterns of alloreactivity can be dictated by single amino acid changes that confer subtle structural differences within the α-helices of the binding cleft. For example, the single amino acid disparity between the HLA-B*4402 and HLA-B*4403 results in graft rejection and graft versus host disease when the donor and recipient are mismatched for these alleles (44, 45), defining them as a “taboo mismatch” in tissue transplantation. HLA-B*0801 and HLA-B*3501 differ by 19 amino acids, and the majority of these differences are not directly accessible by the JL9 TCR. Neverthe-
less, buried polymorphic residues are known to impact on TCR recognition via changes in the local conformation of the α-helices or peptide binding specificity and conformation. The overall structural disparity between HLA B*3501 and HLA B*0801 suggests that either JL9 docks differently between these two pMHC complexes or that the JL9 TCR fortuitously interacts with the regions of structural similarity. Although the MHC typically makes extensive contacts with the TCR, alanine-scan mutagenesis has revealed that only a few MHC residues are critical for engagement (46, 47). The concept of an "energetic hot spot" of recognition was reinforced by the recent structure determination of a "super-bulged" TCR-pMHC complex, where the TCR was observed to make minimal contacts with the MHC heavy chain (48). Of interest, this TCR only made two contact points on the α1 helix (positions 65 and 69) and a small and focused number of contacts on the α2 helix (spanning residues 150–163). Comparative analysis of known TCR-pMHC structures indicated that the residues at positions 65, 69 on the α1 helix, and residue 155 on the α2 helix, represented a "restriction triad," providing a minimal generic footprint for MHC restriction (48). Interestingly, in the allogeneic and cognate pMHC complexes we describe here, the conformation of the triad of residues are conserved structurally (Fig. 6C). Thus, highly focused molecular mimicry in both the C-terminal region of the peptide and the MHC restriction triad of residues provides a plausible explanation for how the JL9 TCR may be able to interact with two such structurally different pMHCs.

The cross-reactivity of TCRs was recently examined in a study on the BM3.3 TCR. These experiments showed that the rearrangements of the complementarity-determining region (CDR) 3α provide an explanation for the inherent cross-reactivity of the TCR (16). For this system, the two MHC molecules are identical, and the authors describe how a single TCR may be able to accommodate peptide variants. Here, the CDR3α loop moves to accommodate changes in the peptide, and as such, the authors propose that it is not molecular mimicry of the peptide but CDR3 loop rearrangement that explains how TCRs may adapt to structurally different peptides. Despite the CDR3α loop rearrangement, the BM3.3 TCR CDR loops focus on regions of structural similarity between the two pMHC ligands (a similar region of the peptide, and regions on the α1 and α2 helices), thus also displaying a degree of focused mimicry.

Molecular mimicry has previously been observed in the cross recognition of an EBV antigen by an autoreactive class II restricted TCR (49). Here the two pMHC ligands are structurally similar, and four TCR-peptide contacts are conserved. The authors hypothesize that structural mimicry of the TCR recognition surface forms a basis for TCR cross-reactivity. The dual specificity of the JL9 TCR for two vastly different peptides and MHC allotypes is another example of such structural mimicry, and as such, JL9 alloreactivity is consistent with a "determinant frequency" alloreponse.

T cells must be able to recognize a vast array of pathogens to provide adequate protection for a host. To cover such an immense number, it is inevitable that TCRs must "wear many hats"; as such, alloreactivity is the price that is paid.

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Structural Insights into T Cell Allorecognition

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