How an alloreactive T-cell receptor achieves peptide and MHC specificity

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T-cell receptor (TCR) allorecognition is often presumed to be relatively nonspecific, attributable to either a TCR focused on major histocompatibility complex (MHC) polymorphisms or the degenerate recognition of allopeptides. However, paradoxically, alloreactivity can proceed with high peptide and MHC specificity. Although the underlying mechanisms remain unclear, the existence of highly specific alloreactive TCRs has led to their use as immunotherapeutics that can circumvent central tolerance and limit graft-versus-host disease. Here, we show how an alloreactive TCR achieves peptide and MHC specificity. The HCV1406 TCR was cloned from T cells that expanded when a hepatitis C virus (HCV)-infected HLA-A2\textsuperscript{a} individual received an HLA-A2\textsuperscript{a} liver allograft. HCV1406 was subsequently shown to recognize the HCV nonstructural protein 3 (NS3):1406–1415 epitope with high specificity when presented by HLA-A2. We show that NS3/HLA-A2 recognition by the HCV1406 TCR is critically dependent on features unique to both the allo-MHC and the NS3 epitope. We also find cooperativity between structural mimicry and a crucial peptide “hot spot” and demonstrate its role, along with the MHC, in directing the specificity of allorecognition. Our results help explain the paradox of specificity in alloreactive TCRs and have implications for their use in immunotherapy and related efforts to manipulate TCR recognition, as well as alloreactivity in general.

T-cell receptor | peptide-MHC | structure | alloreactivity | specificity

T-cell receptor (TCR) recognition of peptides bound and presented by proteins encoded by the major histocompatibility complex (MHC) underlies specificity in cellular immunity. The MHC locus is the most polymorphic region in the human genome, leading to many thousands of expressed MHC variants. Of these MHC variants, any one individual can express, at most, six class I and six class II proteins. Selection processes during thymic education result in a TCR repertoire tolerant of self-peptides presented by self-MHC, as evidenced by the strong immune responses associated with transplantation of tissues with donor/recipient MHC mismatches (alloreactivity). Indeed, precursor frequencies for alloreactive T cells are up to 1,000-fold higher than frequencies for T cells that respond to self-MHC (1). Alloreactive T-cell responses contribute to the significant morbidity associated with rejection of transplanted tissues and graft-versus-host disease.

Surprisingly, many alloreactive T cells display a degree of specificity reflective of traditional syngeneic T cells (2), a counterintuitive finding, given the prevailing theories that alloreactivity is driven by TCR “misfocusing” on non–self-MHC polymorphisms (MHC-centric) or the recognition of a repertoire of unique allopeptides (peptide-centric) (3). Although this enigma has not been fully explained, it has nonetheless led to a growing interest in the use of such “allospecific” TCRs in immunotherapy. Central tolerance mechanisms exist to avoid self-reactivity, hindering the identification of highly avid T cells specific for shared (nonmutated) tumor antigens. The phenomenon of specificity in alloreactivity has thus been exploited to identify T cells capable of recognizing shared tumor antigens with high potency, using antigen-specific T cells from mismatched donors to circumvent tolerance (4, 5). In a related fashion, antigen-specific alloreactive T cells have been used to target conditions such as posttransplantation Epstein–Barr virus-associated lymphoma (6, 7).

Several studies have examined the structural features of allogeneic TCR–pMHC complexes (8–11). An overall assessment of these structures is that rather than demonstrating features that are clearly characteristic of alloreactivity, they illustrate features similar to traditional syngeneic complexes of TCRs with peptides presented by self-MHC (3, 12, 13). The LC13 TCR, for example, was recently shown to engage allo- and self-MHC almost identically, and could distinguish between closely related class I alleles (14, 15). In that case, recognition of an allopeptide occurred via conformational changes that bring it into a conformation mimicking a viral peptide, thus demonstrating the importance of both the MHC and peptide. A recent study of an alloreactive TCR that specifically recognizes an antigen from Hodgkin’s lymphoma concluded that MHC polymorphisms likely determine a unique peptide-binding mode, contributing to high peptide specificity (16). It is clear from these and other studies that specific allorecognition requires elements of the composite peptide-MHC ligand, with the potential for both to influence specificity.

Significance

T-cell alloreactivity drives transplant rejection. Alloreactive recognition is believed to proceed with limited specificity, accounting for the high numbers of alloreactive T cells in humans. Paradoxically, however, many T cells recognize allostrogens with high specificity, and receptors from such T cells are being explored for use in cancer immunotherapy. Here, we explain how a T-cell receptor (TCR) achieves high specificity toward a peptide antigen presented by allo-major histocompatibility complex (MHC). Counter to prevailing theories of alloreactivity, we find that TCR recognition is driven by a cooperative interplay between features unique to both the allo-MHC and the peptide, such that binding is both MHC- and peptide-centric. Our results have broad implications for the determinants of immune recognition and efforts in immunotherapy.

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Data deposition: The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 5Z2D).

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To gain further insight into the paradoxical nature of antigen-specific allovireactivity, we examined an allosreactive human TCR highly specific for the nonstructural protein 3 (NS3):1406–1415 epitope from the hepatitis C virus (HCV) presented by HLA-A2. The HCV1406 TCR was cloned from T cells that expanded when an HCV-infected HLA-A3/A30 individual received an HLA-A2/A24 liver allograft. HLA-A2 and A3/A30 belong to different class I MHC supertypes (17, 18), and A2 has surface properties distinct from HLA-B, HLA-C, nonclassical class I, and the class II proteins. Expression of HCV1406 confers anti-HCV reactivity onto other effector cells, which specifically recognize NS3/HLA-A2 targets independent of the CD8 coreceptor and do not recognize irrelevant targets presented by A2 or other class I MHC proteins (19–22).

In conflict with prevailing theories of T-cell allovireactivity, we show that the specific recognition of NS3/HLA-A2 by HCV1406 is dependent upon three intertwined components: the unique TCR contact surface of A2, including polymorphisms that distinguish it from the other MHC proteins present in the host; the impact of A2 polymorphisms on NS3 peptide binding and conformation; and unique structural and chemical features of the NS3 peptide. Thus, HCV1406 recognition is neither MHC- nor peptide-specific, but is both, emerging from a cooperative interplay of features of the composite peptide-MHC ligand.

Our results help explain the paradox of antigen specificity in allovireactivity and have implications for the use of allospecific TCRs in immunotherapy and related efforts to manipulate TCR recognition.

Results

Fine Specificity of the HCV1406 TCR. To help understand the specificity of the HCV1406 TCR and identify the key residues in the NS3 epitope (sequence KLVALGINAV), we performed a mutational scan of the peptide. We assessed the reactivity of HCV1406 TCR-expressing T cells by cytokine secretion when cocultured with HLA-A2* (referred to as A2) T2 cells pulsed with native NS3:1406–1415 peptide or variants with substitutions at position 1 through 10. We evaluated the relative reactivity of TCR-transduced T cells from two normal human donors as well as TCR-transduced CD8+ or CD8-Jurkat E6.1 cells.

The data from the various cell types displayed similar patterns of reactivity (Fig. 1 A and B), with no recognition of nonpeptide-pulsed targets or targets pulsed with the irrelevant tyrosinase peptide. The response patterns closely resemble the response patterns with the donor-derived T cells in A. (C) Direct binding of the HCV1406 TCR to NS3/A2 measured by SPR. Binding of the TCR to the native epitope yielded a KD value of 16 ± 1 μM. Consistent with the functional data, the K1A substitution substantially weakened binding (KD ≥ 170 μM), and no binding was detected with the irrelevant tyrosinase peptide. RU, resonance units. (D) NS3 peptide binds to A2 with high affinity as measured by DSF (Tm = 63.5 °C). Substitution of the p1 Lys with Ala did not weaken peptide binding (note the y axis is shifted by +4 units for the K1A data to facilitate comparison with the native peptide data).

The HCV1406 TCR Binds NS3/HLA-A2 with a Traditional Binding Mode. We determined the structure of the complex between the HCV1406 TCR and the NS3 peptide presented by A2 at a resolution of 2.5 Å (Table 1). The complex crystallized in space group P3121 with two complexes in the asymmetrical unit. Clear electron density was observed for the TCR–pMHC interfaces in both complexes (Fig. S1). The two complexes were essentially identical: All atoms of the peptides superimposed with an rmsd of 0.8 Å, the A2 backbones superimposed with an rmsd of 0.7 Å, and the TCR backbones superimposed with an rmsd of 0.5 Å. The TCR adopts a typical diagonal orientation over the pMHC, binding directly over the peptide-binding groove with a crossing angle of 30° (Fig. 2 A and B). The incident angle (or tilt, defined as the angle between the plane of the MHC peptide-binding groove and a vertical line down the Va/Vβ pseudosymmetry axis) is 17°. This combination of crossing and incident angles places the docking geometry of HCV1406 near the edge of what has been observed for other A2-binding TCRs (Fig. 2C). However, similar binding angles have been observed with other TCRs (25), and considered alongside the larger set of all human and mouse complexes with class I or class II MHC, the binding geometry of HCV1406 is well within what has been observed for functioning TCRs (Fig. S2). The nondistinctive geometry is particularly clear when it is considered alongside TCRs that bind with “reverse polarity” (26, 27) and the nonsignaling 42F3 TCR in complex with the p3A1 ligand (28). As a further demonstration of its traditional binding geometry, the binding of HCV1046 to NS3/A2 was not distinctive when the position of the center of mass of the Va/Vβ domains over A2 was compared with other A2-binding receptors (Fig. 2D).

Although HCV1406 recognition of NS3/A2 is CD8-independent, the binding geometry is compatible with CD8 binding, because naturally occurring NS3 variants require CD8 for efficient recognition by HCV1406-transduced T cells (20), and as shown in Fig. 1B, the presence of CD8 strengthened the response to single-amino acid NS3 variants.

A total of 2,049 Å2 of solvent accessible surface area is buried in the TCR–pMHC interface, with 54% contributed by Va and 46% by Vβ. The TCR buries 345 Å2 of peptide surface area, or 82% of the peptide surface exposed in the free pMHC complex.

To help understand the specific interactions of the HCV1406 TCR and the NS3 peptide presented by A2 at a resolution of 2.5 Å (Table 1). The complex crystallized in space group P3121 with two complexes in the asymmetrical unit.
The complementarity-determining region (CDR) loops are arranged over the pMHC as commonly observed, with the hypervariable CDR3\[^{\alpha}\]/CDR3\[^{\beta}\] loops over the center of the peptide; the CDR1\[^{\alpha}\] and CDR1\[^{\beta}\] loops over the peptide N- and C-terminal regions, respectively; and the CDR2\[^{\alpha}\] and CDR2\[^{\beta}\] loops over the \(\alpha_2\) and \(\alpha_1\) helices, respectively (Fig. 2B). Using a cutoff distance of 4 Å, there are 134 interatomic contacts in the interface, of which 54 are between the TCR and the peptide and 80 are between the TCR and A2.

**HCV1406 Engagement of the NS3 Peptide.** The HCV1406 TCR engages the NS3 peptide along its entire length, although with greater participation of the N-terminal half and the TCR \(\alpha\)-chain (Fig. 3A and B). The protruding side chains of the peptide are the lysine at p1, the leucine at p5, and the asparagine at p8. The p1 Lys is engaged by Ser33 and Glu34 of CDR1\[^{\alpha}\], which form hydrogen bonds and a salt-bridge with the lysine side chain. The p5 Leu fits into a large hydrophobic cleft formed by residues in CDR1\[^{\alpha}\], CDR3\[^{\alpha}\], and CDR3\[^{\beta}\]. The p8 Asn forms only two contacts with CDR3\[^{\beta}\]. Other contacts are hydrogen bonds made to the peptide backbone at p6 and p7 by Asp102 of CDR3\[^{\alpha}\].

The structural data allow a more detailed interpretation of the specificity and binding experiments shown in Fig. 1. The electrostatic interactions with p1 Lys are clearly crucial for recognition, given that substitution of p1 Lys with alanine is the most detrimental peptide modification studied. The loose accommodation of the p5 Leu side chain in the hydrophobic cleft is consistent with the more moderate impact the p5 Leu\(\rightarrow\)Ala substitution has on recognition. The TCR’s structural bias toward the N-terminal half of the peptide is consistent with the minor impacts of substitutions at positions 8–10 in recognition. The disruption from substitution of the alanine at p6 Gly is attributable to tight packing between the side of the peptide and residues of the A2 \(\alpha_2\)-helix, such that mutation of p6 Gly to anything other than alanine is likely to influence peptide conjugation.

### Table 1. X-ray data collection and refinement statistics

| Data collection |  |
|-----------------|-----------------|
| Resolution, Å   | 37.2–2.50 (2.6–2.5) |
| Space group     | P 31 2 1 |
| Cell dimensions | a, b, c; Å 128.8, 128.8, 223.6 |
| a, b, γ; °     | 90.0, 90.0, 120.0 |
| Unique reflections* | 74,525 (7,352) |
| \(R\)merge     | 0.23 (0.7) |
| \(<I>/\sigma(I)>\) | 9.1 (2.9) |
| Data completeness, % | 99.6 (99.2) |
| Temperature, K  | 100 |
| Wavelength, Å   | 1.0 |
| Refinement statistics |  |
| \(R\)work/\(R\)free \[^{\dagger}\] | 0.20/0.23 |
| No. of protein atoms | 13,339 |
| Rmsd from ideality | 0.015 |
| Bond lengths, Å | 0.015 |
| Bond angles, ° | 0.89 |
| Ramachandran statistics, % |  |
| Favored     | 95 |
| Allowed     | 5 |
| Outlier     | 0.1 |
| Average B factors, Å\(^2\) |  |
| TCR         | 56.7 |
| MHC/\beta2m  | 86.8 |
| Peptide     | 50.6 |
| PDB ID code | 5JZI |

\[^{\dagger}\]R\)free is calculated for a randomly selected 5.0% of reflections not included in refinement.

*Values in parentheses are statistics of the highest resolution shell.

Fig. 2. Overview of the HCV1406–NS3/A2 complex. (A) Structural overview showing the position of the TCR variable domains relative to the pMHC. (B) Position of the CDR3 loops over the A2 molecule. (C) Incident vs. crossing angles for TCRs that bind A2. HCV1406 binds NS3/A2 near the edge as indicated by the red circle, but the docking geometry is within the limits of what has been observed with other receptors (also Fig. S2). (D) Positions of the centers of mass of the Va/Vb domains of the A2-binding TCRs in C over the A2 center of mass, with the center of mass of HCV1406 shown in red.
Comparing the peptide in the TCR–MHC complex with the peptide in the unbound NS3/2 (PDB ID 3MRM) complex indicates that there are no major alterations to peptide confor-

mation upon TCR binding. Interestingly, however, the latter portion of the peptide is “lifted” from the binding groove by 1–1.5 Å upon TCR binding, beginning at p4 Ala and continuing through the C-terminal valine at p10 (Fig. 3C). Peptide lifting is not attributable to weak peptide binding to the MHC, because NS3 binds strongly to A2 as shown above. It may reflect the weaker participation of the β-chain in engaging the peptide, perhaps coupled with weaker interactions of peptides with class I MHC proteins at their C-terminal ends (29).

HCV1406 Engagement of HLA-A2. Although peptide engagement is biased toward the N-terminal half as discussed above, the HCV1406 TCR binds the A2 protein centrally, making contacts along the α1- and α2-helices. On the α1-helix, salt-bridges from Glu34 in CDR1α and Glu101 in CDR3α neutralize the positively charged cluster of Arg65 and Lys66 (Fig. 4 A and C). The Arg65/Lys66 combination is essentially exclusive to A2, and the engagement of this region via electrostatic interactions from CDR1α and CDR3α is a distinctive feature of TCRs that bind A2, influencing the position of the TCR as well as the composition of TCR loops (30).

Further down the α1-helix, the region from Ala69 to Val76 is contacted by CDR3β, whose hairpin structure runs almost parallel to the helix (Fig. 4 B and C). Tyr50 of CDR2β is aligned alongside the helix as commonly observed in many TCR–pMHC complexes (31), contacting Ala69 and Glu72. Glu56 forms a salt-bridge with Arg75, an interaction less frequently observed in TCR complexes but present in several structures of TCRs with A2 (30). Contacts to the α2-helix are more distributed among the TCR-binding loops, with residues in CDR1α, CDR2α, CDR1β, and CDR3β all engaging a span of the helix that ranges from Lys146 in the short arm to Thr163 in the long arm (Fig. 4 D and E). There are multiple electrostatic interactions between the TCR and the length of the α2-helix. These interactions include a salt-bridge from Asp30 of CDR1β to Lys146, which is also observed in other complexes with A2 (30). Another salt-bridge is formed between Lys60 of CDR2α and Glu154, and hydrogen bonds are made to Ala149 and Thr163 from residues in CDR3β and CDR1α, respectively.

Structural Features of Allorecognition: Recognition Is Dependent on Surface Features that Distinguish A2 from Other MHC Proteins in the Host. Although it recognizes NS3 presented by A2, the HCV1406 TCR developed in a host that was HLA-A2+. To gain more insight into the basis for allorecognition of HLA-A2 by HCV1406, we compared the sequences of A2 with the sequences of other class I HLA proteins, emphasizing those amino acids that were contacted by the TCR or otherwise likely to influence receptor binding. Our goal was to isolate any distinctive features of A2 compared with host class I molecules on which HCV1406 may have been positively selected or otherwise engaged during the course of its normal biology, and then to ascertain the extent to which the TCR relied upon these A2-distinctive features for binding. We also assessed the importance of these positions on the peptide–MHC interaction.

We first compared A2 with the classical class I MHC proteins of the allograft recipient, which were typed as A3/A30 and B7/B13 for HLA-A and HLA-B. The HLA-C genes in the host were not recorded, but the C family is less polymorphic and we were able to assess all known C types. Fifty polymorphic sites in human class I MHC proteins are found in the peptide-binding domain. For the A, B, and C alleles, 16 of these alleles are in the α1-helix and seven are in the α2-helix. Most of the polymorphic sites are outside of the contact zone of the TCR. Nonetheless, after examining the sequences in the context of the structure, it was clear that the binding of the HCV1406 TCR is directly influenced by sites that distinguish A2 from the other class I MHC proteins.

The clearest influences of A2 polymorphic sites were seen on the more polymorphic α1-helix (Fig. 5A). Position 62 is a glycine in A2, but a glutamine or arginine in the other A and B proteins.
and in >99% of the C proteins. HCV1406 does not directly contact Glu62, but the position is buried by the CDR1α loop in the structure of the complex, such that a glutamine or arginine would clash with Ser33α and Glu34α (Fig. 5B). Substitution of Glu62 with glutamine or arginine weakened the binding of HCV1406 to N83/A2 between six- and 10-fold as measured by SPR (Fig. 5A and Table 2; data in Fig. S3).

Further down the α1-helix, Arg65 and Lys66 form the cluster of positive charge that is almost exclusive to A2 and helps orient the B7/B13 and C proteins. Mutations at these positions would disrupt the network of electrostatic interactions linking the TCR and its ligand. Substitution of Arg65 with the glutamine found in the B or C proteins, and position 76 is arginine in the other A proteins and isoleucine in the B proteins. In the HCV1406 structure, Arg65 forms salt-bridges with Glu101 of CDR3α and Glu34 of CDR1α, and Lys66 forms a salt-bridge with Glu34α (Fig. 5C). Modeling glutamine in for Arg65 or asparagine/isoleucine in for Lys66 did not introduce any interatomic clashes, but these replacements would disrupt the network of electrostatic interactions linking the TCR and its ligand. Substitution of Arg65 with the glutamine found in the B or C proteins had a dramatic impact on TCR binding, leading to undetectable binding (Table 2). Similar results were obtained when Lys66 was substituted with the asparagine found in A3/A30 or the isoleucine found in B7/B13. The R65Q/K66I double mutation also led to undetectable binding.

Other amino acids that are at least partially distinctive to A2 and are involved in the HCV1406 interface are Ala69, Val76, and Gly79. Position 69 is arginine in the C proteins, position 76 is glutamate in the B7/B13 proteins, and position 79 is arginine in the B7/B13 and C proteins. Mutations at these positions would introduce clashes (A69R), charge repulsion (V76E), or both (G79R) (Fig. S3). As predicted, mutations at these sites also had disruptive effects on TCR binding (Fig. S3).

We identified rare variants of the A, B, and C proteins that had some of the surface features noted above (i.e., C*02:66 has the R65/K66 pair), but none had all. We also examined the less polymorphic nonclassical class I MHC proteins, and found that HCV1406 interacts with elements of the A2 surface that distinguish it from these rare variants as well. For example, variants of the nonclassical class I molecules HLA-E, HLA-F, and HLA-G lack glycines at positions 62 and 79 and the positive charge cluster of Arg65/Lys66 (Fig. 5A).

Although the TCR clearly interacted with sites that are distinctive to A2, it did not show preferential engagement of polymorphic sites at the expense of others, because nonpolymorphic sites made significant contacts, as shown in Fig. 4 (major nonpolymorphic contact sites include Glu72 on the α1-helix and the stretch of Glu154 to Tyr159 on the α2-helix). Moreover, other polymorphic sites did not seem to play a direct role in TCR binding. One example is position 151 on the α2-helix. Position 151 is a histidine in A2, but an arginine in A30 and the B/C proteins. In the structure of the HCV1406 complex, there are contacts between the His151 side chain and the ring of Tyr98 of CDR3β (Fig. 5G). Modeling arginine in the place of His151 would not remove these contacts, or introduce any other clashes. Consistent with the structure, mutation of His151 to arginine had no discernable impact on TCR binding (Table 2 and Fig. S3).

We also compared the sequence of A2 with class II MHC sequences because it is possible that T cells expressing HCV1406 were positively selected on a class II protein. Although class I and class II proteins are structurally homologous, they are sufficiently different at a local level to conclude that A2 presents a surface distinct from any class II MHC proteins in the host. For example, the lengths and twists of the class I/class II peptide-binding groove α-helices differ, as do the identity of the polymorphic and nonpolymorphic sites across the two helices. At a more granular level of detail, no human class II MHC α-chains maintain those features most distinctive to A2 at the equivalent positions in the peptide-binding domain α-helices (e.g., Gly62, Arg65/Lys66).
Overall, our results confirm that binding of HCV1406 to NS3/A2 is strongly dependent upon select polymorphic surface residues that distinguish A2 from other MHC proteins within the host that received the A2 allograft. This distinction includes all known variants of the alleles that were recorded upon transplantation (A3, A30, B7, and B15), as well as the unrecorded HLA-C, nonclassical class I, and class II proteins.

**HLA-A2 Polymorphisms Impact Peptide Binding and Conformation and Indirectly Influence TCR Binding.** The polymorphisms assessed above impacted receptor binding to various extents, but did not significantly impact peptide binding as measured by DSF (Table 2). Due to their positions within the peptide-binding groove, however, the majority of other A2 polymorphisms are expected to impact the MHC–peptide interaction. Impacts on peptide binding are obvious at a high level, because MHC polymorphisms dictate amino acid preferences, particularly at the primary anchors (18). The preferences among A2, A3, A30, B7, B13, and C alleles differ considerably. For example, A2 prefers a small hydrophobic amino acid, such as valine, at the peptide C terminus, whereas A3 prefers a positively charged amino acid, such as lysine. To help quantify the impact of the binding groove polymorphisms on peptide binding, we assessed the binding of the NS3 peptide to the other A, B, and C alleles using the consensus-based MHC–I–binding prediction server from the Immune Epitope Database (32). As expected, the NS3 peptide was predicted to be most strongly bound by A2 (Table S1).

Beyond impacting peptide binding directly, polymorphisms within the binding groove are also likely to influence peptide conformation, detectable as an impact on TCR-binding affinity (33, 34). We tested influences on conformation directly by examining position 152, which is a valine in A2 and B13, but a glutamate or tryptophan in the other A, B, C, and E proteins. Substituting in either the glutamate or the tryptophan is predicted to influence peptide conformation through clashes with Asp8 or Asn8 of the peptide (Fig. 5H). Indeed, replacing Val152 with glutamate weakened TCR binding approximately threefold, whereas replacing it with tryptophan led to much weaker binding (Table 2 and Fig. S3). These outcomes are consistent with alterations in peptide conformation. Of all those mutations tested, these mutations also had the greatest impact on peptide binding to the MHC as assessed by measurements of thermal stability (Table 2). These results confirm that in addition to impacting peptide and TCR binding directly, A2 polymorphisms can impact TCR binding indirectly via their influence on peptide conformation.

**Cross-Reactivity with a Tumor Antigen Reveals Cooperativity Between Structural Mimicry and a Peptide Hot Spot.** In examining the structure of the HCV1406–NS3/A2 complex, we noted that the conformation of the NS3 peptide in the A2-binding groove is remarkably similar to the conformation of the decameric MART-1 tumor antigen (sequence ELAGIGILTV) bound to A2 (35, 36) (Fig. 6A). In addition to the common backbones, except for the p1 residue, there are chemical and structural similarities in both peptide side chains. These similarities are best shown by a superposition of the MART-1 peptide onto the NS3 peptide within the HCV1406–NS3/A2 complex (Fig. 6B). There are no steric clashes with the TCR or MHC, and key residues identified both structurally and functionally show structural and chemical compatibility (e.g., leucine/isoleucine at p5).

In the context of the structure, the only major difference between the two peptides is the p1 Lys in NS3 vs. the p1 Glu in MART-1. The p1 Lys forms multiple electrostatic interactions
with the TCR (Fig. 3), and their importance is demonstrated by the negative impact of the KIA substitution on binding and function (Fig. 1). As shown in Fig. 6B, superimposition of the MART-1 peptide into the HCV1406-NS3/A2 complex showed charge repulsion would occur between the p1 Glu of MART-1 and Glu34 of CDR1α, with a distance between the two carboxylates of only 3.5 Å.

The structural and chemical convergence between NS3 and MART-1, and how they fit within the interface between HCV1406 and A2, prompted us to ask if the HCV1406 TCR could recognize a variant of the MART-1 decamer modified at the N terminus to mimic the interface between HCV1406 and MART-1, with a distance between the two carboxylates of only 3.5 Å.

### Table 2. Impact of mutations at HLA-A2 polymorphic sites on TCR and peptide binding

| HLA-A2 variant | $K_d, \mu M$ | $\Delta \Delta G^\circ, \text{kcal/mol}$ | $T_{me}, ^\circ\text{C}$ |
|---------------|-------------|-------------------------------------|------------------|
| Wild type     | 16 ± 1      | 63.5 ± 0.1                          |
| G65Q          | 100 ± 10    | 1.1                                 |
| G65R          | 164 ± 13    | 1.4                                 |
| R65Q          | nbd§        | >2†                                 |
| K66N          | >300‡       | >2                                  |
| A69R          | 154 ± 8     | 1.3                                 |
| V76E          | nbd         | >2                                  |
| G79R          | 77 ± 2      | 0.9                                 |
| H151R         | 15 ± 1      | 0                                   |
| V152E         | 46 ± 3      | 0.6                                 |
| V152W         | >300‡       | >2                                  | 55.4 ± 0.2

nbd, no binding detected.

§ Change in binding free energy determined from $\Delta \Delta G^\circ = RT\ln(K_d/\text{wild type})$.

¶ Apparent $T_{me}$ of the pMHC complex determined by DSF. Errors are SDs of four experiments.

† Change in $\Delta \Delta G^\circ$ is estimated at greater than 2 kcal/mol based on established SPR measurement sensitivity (40).

Weak binding detected, but not quantifiable. Affinity is estimated at >300 μM.

### Discussion

The drivers of TCR alloreactivity have been variously attributed to a TCR focus on unique allopeptides presented by allo-MHC (peptide-centric) or to a TCR misfocus on polymorphic amino acids on the α-helices of allo-MHC peptide-binding grooves (MHC-centric) (37–39). The surprising specificity of many alloreactive TCRs and structural studies of alloreactive complexes have challenged these theories (3, 12, 13). Highly specific alloreactive T cells and TCRs are being explored for targeting what otherwise would be poorly immunogenic shared tumor antigens and have been adopted for treatment of posttransplantation-associated lymphoproliferative disease (4, 6, 7).

Here, we show that for the highly specific alloreactive TCR HCV1406, recognition is both peptide- and MHC-centric, with polymorphisms impacting how the TCR sees the MHC protein as well as how the peptide is bound and presented. The structure of the HCV1406-NS3/A2 complex revealed that the TCR engages with a traditional binding mode, geometrically near the limits but still within what has been observed for other TCR complexes with A2. The TCR contacts polymorphic and non-polymorphic sites along the α-helices of the A2-binding groove, but does not focus solely on polymorphic sites, nor does it avoid them. Of note are how the TCR interfaces with features that are unique to A2, most notably the cluster of positive charge composed of arginine at position 65 and lysine at position 66. Electrostatic interactions with Arg65 and Lys66 are a characteristic signature of TCRs that bind A2 (30), and changing Arg65 or Lys66 to their counterparts in other human class I MHC proteins disrupts receptor binding without impacting peptide binding. Recognition is likewise influenced by other A2-distinctive features, such as the collective identity of positions 62, 69, 76, and 79. Thus, binding of HCV1406 is dependent upon features that distinguish the TCR-interacting surface of A2 from other MHC proteins, both classical and as well as class II. As seen in other systems (33, 34), other polymorphic sites within the A2 peptide-binding groove impact alloreactivity through influences on peptide selection as well as peptide conformation. Recognition also depends upon details of the NS3 peptide that are not influenced by polymorphisms. The defining example is the lysine at p1, which is engaged by residues of CDR1α and whose replacement with alanine substantially weakens binding and eliminates functional recognition.

Overall, the picture that emerges is that alloreactive recognition of NS3/A2 by HCV1406 is driven by the following elements that together, define the composite peptide/MHC surface: (i) the unique TCR contact surface of A2, including polymorphisms that distinguish it from other MHC proteins that HCV1406 may have encountered in its host; (ii) properties of the NS3 peptide that are influenced by polymorphisms within the peptide-binding groove; and (iii) structural and chemical properties of the NS3 peptide itself. Perturbation of any one of these elements alone is sufficient to weaken receptor binding substantially.

These results explain the allospecific nature of HCV1406, but also their lessons for alloreactivity in general. Our findings are consistent with fundamental studies on TCR recognition showing that TCR engagement of pMHC depends on unique surface chemistry provided by both the peptide and MHC, whose “roles” in binding are not easily separable (40). Our findings therefore echo suggestions that at the molecular level, TCR allorecognition is more “normal” than “abnormal” (3, 12). We do not argue that more fully peptide- or MHC-centric alloreactivity does not exist, but that perhaps we should not anticipate TCR allorecognition to be unusually distinctive. If such is the case, our results may also help explain the high frequency of alloreactive T cells, because the combination of different surface chemistries on allo-MHC, the influence of polymorphisms on peptide selection and conformation, and the presentation of unique peptides collectively provide for recognition surfaces quite distinctive from the recognition surfaces found syngeneically.

Our findings also offer perspectives on the determinants of TCR MHC restriction. MHC restriction has been proposed to emerge from the requirements for the CD4/CD8 coreceptors during thymic selection and T-cell signaling (41). Our data make it clear that if thymic education does select for MHC restriction, it is sufficiently “fuzzy” to permit TCR engagement of allo-MHC in standard geometries. Alloreactive TCRs such as HCV1406 that bind in standard geometries could underscore a need to form a competent TCR-pMHC signaling complex that is structurally compatible with the binding of coreceptor and/or CD3 signaling units (42). There is evidence that such structural requirements do
exist: In one instance, a TCR–pMHC complex with a highly unusual geometry did not signal (28), and, more recently, TCRs that bind with reverse polarity have been shown to lead to poor T-cell activation (26).

On the other hand, MHC restriction has been proposed to emerge from an intrinsic bias of TCRs toward MHC proteins (43), and there is evidence that TCR genes have coevolved with genes of the MHC (44). Our observations of a traditional binding mode with associated specificity could therefore reflect the influence of coevolved TCR-MHC contacts in binding. Indeed, the HCV1406–NS3/A2 complex displays the hallmarks of TCRs that bind A2, most notably the accommodation of the positive charges on Arg65 and Lys66. We recently presented evidence that, given the high frequency of A2 in human populations, the need to offset this charge cluster has influenced the composition of TCR genes, leading to biased recognition and restricted TCR positioning (30). Therefore, although geometrical requirements for coreceptor and CD3 compatibility could influence HCV1406 binding, the complex displays features associated with TCR-MHC coevolution and a subsequent inherent bias of TCRs for MHC proteins.

The fact that we could generate an HCV1406 cross-reactive peptide based not on the NS3 viral epitope but on the structural properties of the unrelated MART-1 tumor epitope underscores the importance of structural mimicry (as opposed to sequence mimicry) in T-cell recognition, both natural and allo. The observation that HCV1406 can cross-react between the NS3 epitope and a single amino acid variant of MART-1 may seem coincidental: Of the billions of possible decameric peptides, why would the well-studied MART-1 peptide be identified as the root of a molecular mimic? First, we note that in the central regions, MART-1 and NS3 are both rather featureless, lacking bulky or polar/charged side chains. Second, the backbone conformations of the peptides are nearly identical, which is not unusual with 9- and 10-mers bound to class I MHC proteins. Third, where the peptides are most different (p1 Lys in NS3 and p1 Glu in MART-1) is the exact region where substitutions have the greatest impact and direct cross-recognition. HCV1406 cross-reactivity with a modified MART-1 peptide therefore underscores the importance of hot spots in TCR specificity: The TCR engages a key region of the peptide that most directly influences specificity, restricting amino acid composition there but leaving other regions open to more diversity (45, 46). Our observations illustrate how hotspots can work alongside mimicry in directing TCR specificity/cross-reactivity and reinforce how peptide features working alongside the distinctive surface chemistry of allo-MHC can drive allospecificity.

In conclusion, we have shown that the specificity of the HCV1406 TCR emerges from how the TCR engages the cooperative pMHC surface, with MHC polymorphisms influencing receptor binding directly via TCR-MHC contacts and indirectly via impacts on peptide binding and conformation. These observations can explain the paradox of TCR specificity in alloreactivity, and provide a structural and molecular context for the use of alloreactive TCRs in immunotherapy. More generally, our observations sharpen the discussion of how MHC restriction is achieved, and provide a clear example of how a peptide hotspot combined with structural mimicry can drive TCR cross-reactivity.

Methods

Cells, Media, and Reagents. The 293GP, PG13, T2, and Jurkat cell lines were obtained from the American Type Culture Collection. The 293GP cells were

the structural predictions, in a direct binding experiment, HCV1406 recognizes a variant of MART-1 with the p1 Glu substituted with lysine. No recognition was seen of the native MART-1 peptide, emphasizing the importance of the p1 Lys. Binding to NS3 from Fig. 1 is shown as a dashed line for comparison. (D) E1K-modified MART-1 peptide was also recognized in a functional assay by HCV1406-transduced CD8+ T cells, as shown in this titration assessing IFN-γ release.
maintained in DMEM supplemented with 10% FBS. The PG13 cells were maintained in Iscove's DMEM supplemented with 10% FBS. The T2 and Jurkat cell lines were maintained in RPMI medium 1640 supplemented with 10% FBS.

Generation of CD8+ Jurkat cells has been described previously (19, 47). Briefly, a modified SAMEN retroviral vector containing full-length human CD8α and β, separated by an internal Ssr promoter, and an internal ribosome entry site/neo cassette was used to transduce cells. CD8+ cells were sorted for high and uniform expression by FACS using anti–CD8-PerCPCy5.5 mAb and maintained in RPMI medium 1640 supplemented with 10% FBS and 5% FBS, G418.

Human T cells were derived from deidentified apheresis products from normal, healthy donors, purchased from Key Biologics. Ficol-Hypaque density gradient centrifugation was used to isolate peripheral blood mononuclear cells (PBMCs). Activated T cells used for retrotransduction were generated by stimulating PBMCs with 50 ng/mL anti-CD3 mAb for 3 d in AIM-V medium supplemented with 5% heat-inactivated pooled human AB serum, 300 IU/mL recombinant human IL-2, and 100 ng/mL recombinant human IL-15.

As described in the original publication describing the HCV1406 TCR, HLA typing was performed using PCR amplification with sequence-specific primers (21). The original publication did not list the B types, but they were recorded at the time as HLA-B7 and HLA-B13.

Retroviral Transduction. Retroviral supernatants were prepared using a high-titer stable retroviral producer cell line, PG13, expressing HCV1406 TCR in a modified SAMEN retroviral vector. Retroviral vectors contained the TCR α-chain linked to the TCR ρ-chain and a truncated CD34 molecule by P2A and T2A self-clearing sequences, respectively. Generation of these stable producer cell lines and collection of retrovirus have been described previously (20, 22).

Jurkat cells, CD8+ Jurkat cells, and anti-CD3-activated primary T cells were transduced to express HCV1406 TCR via spinoculation, as previously described (20, 22, 47). Briefly, 0.5 mL per well of 30 μg/mL retinogen was used to coat 24-well, flat-bottom, nontissue culture-treated plates overnight at 4 °C. The next day, plates were blocked with 0.5 mL per well of 2% PBSA (PBS containing 2% BSA in PBS for 30 min at room temperature and washed with 2 mL per well of PBS). Plates were loaded with 2 mL per well of filtered retroviral supernatant, spun for 2 h at 2,000 × g at 32 °C, and gently aspirated. Two million Jurkat cells in 1 mL of RPMI/10% FBS or 2 million OKT3-activated T cells in 1 mL of AIM-V supplemented with 5% human immunoglobulin monoclonal antibody (hAB), 600 IU/mL recombinant human IL-2 (rHIL-2), and 200 ng/mL rhIL-15 were mixed with 1 mL per well of retroviral supernatant and added to each well. The plates were spun again for 2 h at 2,000 × g at 32 °C. After 24 h, the transduced cells were harvested and transfected to tissue culture-treated flasks. Three days later, the cells were analyzed for transduction efficiency by FACS analysis using anti-CD34-phycocerythrin (PE) mAb. T cells or Jurkat cells were then sorted for TCR-transduced cells by positive selection using anti-CD34 mAb-coated immunomagnetic beads. TCR-transduced T cells were also sorted into CD4+ or CD8+ fractions using anti-CD4 and anti-CD8 mAb-coated immunomagnetic beads.

Immunofluorescence and Cytokine-Release Assays. HCV1406-TCR transduced Jurkat cells and T cells were evaluated for transduction efficiency via immunofluorescence staining and quantified via flow cytometry as previously described (20, 22). The mAbs used in these experiments were anti-CD3-APC/Cy7, anti-CD4-PE/Cy7, anti-CD8-PerCP/Cy5.5, and anti-CD34-PE. Flow cytometry was performed using a Canto II flow cytometer, and data were analyzed with FlowJo (FlowJo, LLC).

Antigen reactivity by HCV1406 TCR-transduced T cells and Jurkat cells against peptide-loaded T2 cells was assessed by cytokine release assays as previously described (49). Briefly, T2 cells were pulsed with 10 μg/mL peptide for 2 h. For peptide titrations, T2 cells were pulsed with peptide concentrations ranging from 10-6 to 0.001 μg/mL. Target T2 (1 × 105) cells and effector (TCR-transduced T cells or Jurkat; 1 × 105) cells were cocultured in a 1:1 ratio in 96-well, U-bottom, tissue culture plates in 200 μL of RPMI 1640 supplemented with 10% FBS. Phorbol 12-myristate 13-acetate (10 ng/mL) was added to Jurkat cell cocultures to enhance sensitivity of stimulation as described elsewhere (20, 22). Cocultures were incubated at 37 °C overnight, supernatants were harvested, and the amount of IFN-γ or IL-2 released by 1 × 105 T cells or Jurkat cells, respectively, was measured by ELISA.

Peptides and Proteins. Soluble pHMC and TCR were refolded from bacterially expressed inclusion bodies following established procedures (50). Briefly, inclusion bodies of HLA-A2 heavy chain, β2m, TCR α-chain, and TCR β-chain were dissolved in 8 M urea. For MHC refolding, HLA-A2 heavy chain and β2m were diluted into MHC refolding buffer containing 100 mM Tris HCl (pH 8.3), 400 mM L-arginine, 6.3 mM cysteamine, 3.7 mM cystamine, 2 mM EDTA, and 100 μg/mL P2A and T2A molecules at 21°C for 1 h. Refolded complexes were purified with anion exchange chromatography followed by size exclusion chromatography. Peptides were synthesized commercially by Synthetic Biopolymers or Genscript, and were obtained at 95% purity. Mutations in the HLA-A2 heavy chain were made using PCR-based mutagenesis.

Peptide-binding predictions were performed using the Immune Epitope Database analysis resource Consensus tool (32) which combines predictions from ANN (51–53), SMMP (54), and Combibl (55). HLA sequences were compared using data from the IMGT database (56).

Protein Crystalization, Data Collection, Structure Refinement, and Analysis. Crystals of the HCV1406–NS3/2A complex were grown in 13% vol/vol PEG 3350, 0.1 M sodium cacodylate (pH 6.1), 0.2 M ammonium sulfate, and 3% w/vol 1,5-diaminopentane dihydrochloride at a protein concentration of 6 mg/mL at 20 °C. Crystallization was performed by hanging drop vapor diffusion.

Cryo-pretreatment, crystals were transferred into 20% glycerol/80% mother liquor for 30 min and immediately frozen in liquid nitrogen. Diffraction data were collected at 1.5°C, using a CMOS in the Advanced Photon Source (Advanced Photon Laboratories). Data were indexed, integrated, and scaled with the program XDS22. The complexes were solved by molecular replacement with Phaser (57) using the Protein Database (PDB) ID codes 3QEU and 3MRM as search models (58). Rigid body refinement followed by translation/libration/screw refinement was performed with PHenix and Refmac5 (59, 60). The twinning law h-h, k-k, l-l was determined by Xtriage and applied in the last round of refinement. Evaluation of the models and fitting to maps were performed with the program Coot. The model was checked in WHATIF and MolProbity to evaluate the structure during the refinement (61, 62). Atomic positioning was verified with a simulated annealing composite OMIT map calculated in Phenix. Density for the α3-domain of HLA-A2 was weak for both molecules in the asymmetrical unit, contributing to greater than typical Ramachandran deviations. The structure has been deposited in the PDB with ID code S1Z. Analysis of hydrophobic bonds and van der Waals contacts was performed with the PISA server, using a cutoff of 4 Å for contacts (63). Solvent-accessible surface areas were calculated using Discovery Studio with a probe radius of 1.4 Å. Superimpositions and modeling of clashes and compatibility were performed with Discovery Studio. TCR crossing and incipient angles were calculated as previously described (64, 65).

SPR and DSF. Steady-state–binding experiments were performed on Biacore 3000 and 7200 instruments in HBS-EP buffer containing 10 mM HEPES (pH 7.4), 150 mM NaCl, 3 mM EDTA, and 0.005% surfactant P20. The HCV1406 TCR was covalently coupled to a CMS sensor chip via standard amine coupling. Equilibrium-binding experiments were performed by injecting 25 μL of mHMC at a flow rate of 5 μL/min over the concentration range of 0.5 to 200 μM. The responses at equilibrium were determined by averaging the peak signal over the final 10 s of the injection period and subtracting the responses from identical injections over a blank flow cell. Experiments were performed at 25 °C. Data were processed with BioEvaluation 4.1 and fit with OriginPro using a 1:1 binding model. For each experiment, all injections were repeated twice and the two datasets were fit globally (66). Each experiment was performed in triplicate, and the results were averaged for reporting values and errors.

DSF was performed as previously described (24), using an Applied Biosystems StepOnePlus RT-PCR instrument with the excitation and emission wavelengths set to 587 nm and 607 nm, respectively. Protein was denatured in 10 mM HEPES (pH 7.4), 150 mM NaCl, 3 mM EDTA, and 0.005% surfactant P20 at concentrations of 4 μM, 6 μM, 8 μM, and 10 μM. SYPRO orange (Invitrogen) was added at fivefold and 10-fold concentrations to each protein concentration with a total reaction volume of 20 μL. The temperature range spanned 25–95 °C with a scan rate of 1 °C/min. Data analysis was performed in OriginPro 9.0. Apparent Tm values were determined from the first derivative of the melting curve. The derivative curve was processed with the single-peak-fitting algorithm in OriginPro, fitting the peak to a Gaussian function. The apparent Tm values were averaged for measurements at four concentrations. Errors are reported as the SD of the four measurements.

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1. Suchin EJ, et al. (2001) Quantifying the frequency of alloreactive T cells in vivo: New answers to an old question. J Immunol 166:973-981.

2. Smith PA, Brunmark A, Jackson MR, Potter TA (1997) Peptide-independent recognition by alloreactive cytotoxic T lymphocytes (CTL). J Exp Med 185:1023–1033.

3. Felix NI, Allen PM (2007) Specificity of T-cell alloreactivity. Nat Rev Immunol 7:942-953.

4. Kumar S, et al. (2014) Alloreactive cytotoxic T cells provide means to decipher immunopeptide and reveal a plethora of tumor-associated self-epitopes. Proc Natl Acad Sci USA 111:403–408.

5. Sadovnikova E, Staus HJ (1996) Peptide-specific cytotoxic T lymphocytes restricted by nonself major histocompatibility complex class I molecules: reagents for tumour immunotherapy. Proc Natl Acad Sci USA 93:13114–13118.

6. Barker JN, et al. (2010) Successful treatment of EBV-associated posttransplantation lymphoma after cord blood transplantation using third-party EBV-specific cytotoxic T lymphocytes. Blood 116:5045-5049.

7. Cooper LN (2010) Off-the-shelf T-cell therapy. Blood 116:4741–4743.

8. Colf LA, et al. (2007) How a single T cell receptor recognizes both self and foreign MHC. Cell 129:135–146.

9. Siracusa LC (2005) Structural basis of plasticity in T cell receptor recognition of a self-peptide-MHC antigen. Science 279:1166–1172.

10. Heenekke J, Wiley DC (2002) Structure of a complex of the human alphabeta T cell receptor (TCR) HA1.7, influenza hemagglutinin peptide, and major histocompatibility complex class II molecule, HLA-DR4 (DRA*0101 and DRB1*0401): insight into TCR cross-restriction and alloreactivity. J Exp Med 195:571–581.

11. Reiser JB, et al. (2003) CD3δ loop flexibility contributes to the degeneracy of TCR recognition. Nat Immunol 4:241–247.

12. Ely LB, Burrows SR, Purcell AW, Rossjohn J, McCluskey J (2008) T cells behaving badly: Structural insights into alloreactivity and autoimmunity. Curr Opin Immunol 20:575–580.

13. Archbold JK, Macdonald WA, Burrows SR, Rossjohn J, McCluskey J (2008) T cell alloreactivity: A case of mistaken identity or deja vu? Trends Immunol 29:220–226.

14. Macdonald WA, et al. (2009) T cell allorecognition via molecular mimicry. Immunity 31:897–908.

15. Kjer-Nielsen L, et al. (2003) A structural basis for the selection of dominant alphabeta T cell receptors in antiviral immunity. Immunity 18:53–64.

16. Simpson AA, et al. (2011) Structural and energetic evidence for highly peptide-specific tumor antigen targeting via allo-MHC restriction. Proc Natl Acad Sci USA 108:21176–21181.

17. Harjo AN, Sng LFP, Tong JC (2016) Clustering HLA class I superalleles using structural bioinformatics. PLoS One 9:e86655.

18. Sidney J, Peters B, Frahm N, Brandr C, Sette A (2008) HLA class I superfamilies: A revised and updated classification. BMC Immunol 9:1.

19. Callender GG, et al. (2006) Identification of a hepatitis C virus-reactive T cell receptor that does not require CD8 for target cell recognition. Hepatology 43:973–981.

20. Spear TT, et al. (2016) Hepatitis C virus-cross-reactive T cell receptors. J Leukoc Biol 100:545–557.

21. Rosen HR, et al. (2004) Cutting edge: Identification of hepatitis C virus-specific CD8 T cells restricted by donor HLA alleles following liver transplantation. J Immunol 173:5355–5359.

22. Spear TT, et al. (2016) TCR gene-modified T cells can efficiently treat established hepatitis C-associated hepatocellular carcinoma tumors. Cancer Immunol Immunother 65:293–304.

23. Laugel B, et al. (2007) Different T cell receptor affinity thresholds and CD8 coreceptor dependence govern cytotoxic T lymphocyte activation and tetramer binding properties. J Biol Chem 282:23379–23386.

24. Hellman LM, et al. (2016) Differential scanning fluorimetry based assessments of the thermal and kinetic stability of peptide-MHC complexes. J Immunol Methods 432:95–101.

25. Birnbaum ME, et al. (2014) Deconstructing the peptide-MHC specificity of T cell receptor-transduced cells. Cancer Res 66:11455–11461.

26. Clay TM, et al. (1999) Efficient transfer of a tumor antigen-reactive TCR to human peripheral blood lymphocytes confers anti-tumor reactivity. J Immunol 163:507–513.

27. Zacharias M, Springer S (2004) Conformational flexibility of the MHC. Annu Rev Immunol 22:271–281.

28. Adams JJ, et al. (2011) T cell receptor signaling is limited by docking geometry to human class I major histocompatibility complex. J Immunol 187:2931–2938.

29. Zacharias M, Springer S (2004) Conformational flexibility of the MHC. Annu Rev Immunol 22:271–281.

30. Heenekke J, Wiley DC (2002) Structure of a complex of the human alphabeta T cell receptor (TCR) HA1.7, influenza hemagglutinin peptide, and major histocompatibility complex class II molecule, HLA-DR4 (DRA*0101 and DRB1*0401): insight into TCR cross-restriction and alloreactivity. J Exp Med 195:571–581.

31. Reiser JB, et al. (2003) CD3δ loop flexibility contributes to the degeneracy of TCR recognition. Nat Immunol 4:241–247.

32. Ely LB, Burrows SR, Purcell AW, Rossjohn J, McCluskey J (2008) T cells behaving badly: Structural insights into alloreactivity and autoimmunity. Curr Opin Immunol 20:575–580.

33. Archbold JK, Macdonald WA, Burrows SR, Rossjohn J, McCluskey J (2008) T cell alloreactivity: A case of mistaken identity or deja vu? Trends Immunol 29:220–226.

34. Macdonald WA, et al. (2009) T cell allorecognition via molecular mimicry. Immunity 31:897–908.

35. Kjer-Nielsen L, et al. (2003) A structural basis for the selection of dominant alphabeta T cell receptors in antiviral immunity. Immunity 18:53–64.

36. Simpson AA, et al. (2011) Structural and energetic evidence for highly peptide-specific tumor antigen targeting via allo-MHC restriction. Proc Natl Acad Sci USA 108:21176–21181.

37. Harjo AN, Sng LFP, Tong JC (2016) Clustering HLA class I superalleles using structural bioinformatics. PLoS One 9:e86655.

38. Sidney J, Peters B, Frahm N, Brandr C, Sette A (2008) HLA class I superfamilies: A revised and updated classification. BMC Immunol 9:1.

39. Callender GG, et al. (2006) Identification of a hepatitis C virus-reactive T cell receptor that does not require CD8 for target cell recognition. Hepatology 43:973–981.

40. Spear TT, et al. (2016) Hepatitis C virus-cross-reactive T cell gene-modified T cells: A model for immunotherapy against diseases with genomic instability. J Leukoc Biol 100:545–557.

41. Rosen HR, et al. (2004) Cutting edge: Identification of hepatitis C virus-specific CD8 T cells restricted by donor HLA alleles following liver transplantation. J Immunol 173:5355–5359.

42. Spear TT, et al. (2016) TCR gene-modified T cells can efficiently treat established hepatitis C-associated hepatocellular carcinoma tumors. Cancer Immunol Immunother 65:293–304.

43. Laugel B, et al. (2007) Different T cell receptor affinity thresholds and CD8 coreceptor dependence govern cytotoxic T lymphocyte activation and tetramer binding properties. J Biol Chem 282:23379–23386.

44. Hellman LM, et al. (2016) Differential scanning fluorimetry based assessments of the thermal and kinetic stability of peptide-MHC complexes. J Immunol Methods 432:95–101.

45. Birnbaum ME, et al. (2014) Deconstructing the peptide-MHC specificity of T cell recognition. Cell 157:1073–1087.

46. Grass S, et al. (2016) Reversed T cell receptor docking on a major histocompatibility class I complex limits involvement in the immune response. Immunity 45:749–760.

47. Beringer DX, et al. (2010) T cell receptor reversed polarity recognition of a self-antigen major histocompatibility complex. Nat Immunol 11:1563–11561.

48. Adams JJ, et al. (2011) T cell receptor signaling is limited by docking geometry to peptide-major histocompatibility complex. Immunity 35:681–693.

49. Zacharias M, Springer S (2004) Conformational flexibility of the MHC class I alpha1alpha2 domain in peptide bound and free states: A molecular dynamics simulation study. Biophys J 87:2203–2214.

50. Blevins SJ, et al. (2016) How structural adaptability exists alongside HLA-A2 bias in the human αβ TCR repertoire. Proc Natl Acad Sci USA 113:E1276–E1285.

51. Marrack P, Scott-Browne DJ, Dai S, Gaspin L, Kappler JW (2008) Evolutionarily conserved amino acids that control TCR-MHC interaction. Annu Rev Immunol 26:171–203.

52. Fiers W, et al. (2017) The immune epitope database and analysis resource in epitope discovery and vaccine design. Front Immunol 8:278.

53. Macdonald WA, et al. (2003) A naturally selected dimorphism within the HLA-B44 supertypes alters class I structure, peptide repertoire, and T cell recognition. J Exp Med 198:679–691.