T Cell Allorecognition via Molecular Mimicry

Whitney A. Macdonald,1 Zhenjun Chen,2 Stephanie Gras,1 Julia K. Archbold,1 Fleur E. Tyran,1 Craig S. Clements,1 Mandvi Bharadwaj,2 Lars Kjer-Nielsen,2 Philippa M. Saunders,2 Matthew C.I. Wilce,1 Fran Crawford,4 Brian Stadinsky,4 David Jackson,2 Andrew G. Brooks,2 Anthony W. Purcell,3 John W. Kappler,4 Scott R. Burrows,5 Jamie Rossjohn,1,6,* and James McCluskey2,6,*

1The Protein Crystallography Unit, Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria 3800, Australia
2Department of Microbiology & Immunology
3Department of Biochemistry and Molecular Biology and Bio21 Molecular Science and Biotechnology Institute University of Melbourne, Parkville, Victoria 3010, Australia
4Howard Hughes Medical Institute, Integrated Department of Immunology, National Jewish Health, 1400 Jackson Street, Denver, CO 80206, USA
5Cellular Immunology Laboratory, Queensland Institute of Medical Research and Australian Centre for Vaccine Development, Brisbane, 4029, Australia
6These authors contributed equally to this work
*Correspondence: jamie.rossjohn@med.monash.edu.au (J.R.), jamesm1@unimelb.edu.au (J.M.)
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SUMMARY

T cells often alloreact with foreign human leukocyte antigens (HLA). Here we showed the LC13 T cell receptor (TCR), selected for recognition on self-HLA-B∗0801 bound to a viral peptide, alloreacts with B44 allotypes (HLA-B∗4402 and HLA-B∗4405) bound to two different allopeptides. Despite extensive polymorphism between HLA-B∗0801, HLA-B∗4402, and HLA-B∗4405 and the disparate sequences of the viral and allopeptides, the LC13 TCR engaged these peptide-HLA (pHLA) complexes identically, accommodating mimicry of the viral peptide by the allopeptide. The viral and allopeptides adopted similar conformations only after TCR ligation, revealing an induced-fit mechanism of molecular mimicry. The LC13 T cells did not alloreact against HLA-B∗4403, and the single residue polymorphism between HLA-B∗4402 and HLA-B∗4403 affected the plasticity of the allopeptide, revealing that molecular mimicry was associated with TCR specificity. Accordingly, molecular mimicry that is HLA and peptide dependent is a mechanism for human T cell alloreactivity between disparate cognate and allogeneic pHLA complexes.

INTRODUCTION

Clonally distributed αβ T cell receptors (TCR) corecognize specific antigenic peptides bound to polymorphic human leukocyte antigens (HLA) of the major histocompatibility complex (MHC) (Davis et al., 1998; Rudolph et al., 2006). HLA polymorphism ensures that the HLA molecules from different haplotypes can bind a broad sample of self and microbial peptide antigens necessary to mediate adaptive immunity (Parham and Ohta, 1996). Developing T cells in the thymus are selected for weak recognition of one or more of the many self-peptide-HLA complexes (Bevan and Hünig, 1981; Hogquist et al., 1993) generating a large repertoire of T cells, each expressing individual TCRs (Fink and Bevan, 1995). Inherent structural plasticity of the TCR contributes to chance improvements in recognition of novel peptide-HLA complexes (pHLA) that are generated when self-peptides are replaced with foreign peptides during infection (Garcia et al., 1998, 1999; Rudolph et al., 2006). This recognition triggers effector immunity by responsive T cells.

Despite pHLA diversity and TCR plasticity, αβ-T cell responses remain exquisitely specific (Archbold et al., 2009) and are developmentally restricted to recognizing host (self) HLA (Jameson et al., 1995; Zinkernagel and Doherty, 1974), with the exception of minor subpopulations like NKT cells (Borg et al., 2007). This “genetic restriction” of MHC-directed T cell immunity means that T cells recognize only cognate antigen presented by one of the host HLA molecules in which they developed (also termed MHC restriction) (Zinkernagel and Doherty, 1974). This “law” of immunology is a defining paradigm of antigen-specific T cell immunity (Garboczi and Biddison, 1999).

Surprisingly, some T cells break the “law” of MHC restriction (Sherman and Chattopadhyay, 1993) by directly reacting with “foreign” HLA molecules from unrelated (allogeneic) individuals. HLA polymorphism involving just one amino acid, or up to 30 or more residues, can induce an immune response toward transplanted cells, the severity of which is variable. Thus, some HLA mismatches lead to worse transplant outcomes than others, so-called taboo mismatches (Doxiadis et al., 1996; Kawase et al., 2007). For instance, mismatching across closely related HLA allotypes such as HLA-B∗4402 and HLA-B∗4403 provokes vigorous T cell alloreactivity (Mifsud et al., 2008) associated with transplant rejection (Fleischhauer et al., 1990) and acute graft-versus-host disease (Keever et al., 1994) after haemopoietic stem cell transplantation, despite the broadly similar peptide repertoires of these allotypes (Macdonald et al., 2003). In contrast, highly divergent HLA mismatches may paradoxically have a better outcome in some transplant settings (Heemsbergen et al., 2007). Regardless, T cell alloreactivity is responsible for much of the morbidity and mortality associated with tissue transplantation, including graft-versus-host disease (Afzali et al., 2007).
RESULTS

Peptide-Dependent Alloreactivity of LC13 T Cells

To investigate the molecular basis of natural human T cell alloreactivity, we examined the prototypic TCR termed LC13 that recognizes the immunodominant HLA-B*0801-restricted epitope, FLRGRAYGL from EBNA 3A of Epstein-Barr virus (EBV) (Argaet et al., 1994; Burrows et al., 1994). LC13 also allorreacts with HLA-B*4402 and HLA-B*4405, related allolotypes that differ from each other by only one residue but differ from HLA-B*0801 by 24 and 25 amino acids, respectively.

Alloreactivity can be either dependent or independent of the HLA-bound peptide (Heath et al., 1989, 1991; Smith et al., 1997a, 1997b). Therefore, we examined whether LC13 allorecognition of HLA-B*4405 required a specific peptide(s). Presentation of the HLA-B*4405 alloligand was examined in transfecants of the class-I HLA-deficient mutant lymphoblastoid cell line (CLL) C1R and the TAP-deficient T2 cell line (Alexander et al., 1997). The C1R.B*4405 cells, but not the parental C1R cells, were lysed by LC13 cytotoxic T-lymphocyte (CTL) indicating constitutive presentation of an allogeneic ligand by these cells (Figure 1A). However, coexpression of the viral TAP inhibitor ICP47 essentially abolished allorecognition of C1R-B*4405 by LC13 (Figure 1A), indicating TAP dependence of this allogeneic ligand. Exogenous loading of C1R-B*4405-ICP47 cells with viral peptide restored recognition by an antiviral CTL clone (DM1) (Archbold et al., 2009) but did not restore killing by LC13 CTL (Figures 1A and 1B). The T2.B*4405 cell line was not recognized by the human T cell line Jurkat coexpressing the LC13.B*4405 TCR and human CD8a/B genes (LC13.Jurkat) (Beddoe et al., 2009). Stabilization of “empty” HLA-B*4405 molecules with a HLA-B*4405-binding peptide (DP2 peptide) did not sensitize the T2.B*4405 cells for recognition by LC13.Jurkat (Figure 1C). Notably, the T2.B*0801 and C1R.B*0801 cell lines loaded with exogenous FLRGRAYGL viral peptide (“virotope”) activated LC13.Jurkat (Figures 1C and 1D), as did C1R.B*4402 and C1R.B*4405 transfecants (Figure 1D). Collectively, these data indicate that the alloreactivity of the LC13 TCR behaved in a peptide-dependent manner.

Identification of a Candidate Allopeptide Presented by HLA-B*4405

A major hurdle in understanding the basis of alloreactivity is the identification of authentic antigenic peptides (the allopeptides) bound to the allogeneic HLA molecule. Murine examples of allolreactive T cells have been the most informative to date, including the allolreactive BM3.3 TCR (Reiser et al., 2000) and the 2C TCR (Coff et al., 2007; Speir et al., 1998) for which pMHC allopeptide structures are solved. However, pathogen-derived cognate ligands for the BM3.3 and 2C T cells remain unknown.

To identify a candidate LC13 allopeptide(s), we generated insect cells expressing individual baculoviral constructs from a library of HLA-B*4405 molecules covalently complexed with randomized peptides. Infected insect cells were screened for interaction with recombinant, bivalent LC13 TCR (Crawford et al., 2006). Repeated rounds of sorting allowed expansion of HLA-B*4405-positive cells expressing a ligand that bound LC13 TCR (Figure 2A). Peptide insert sequences were obtained from 36 positive clones with 30 of these encoding the peptide EEYLKAWTF. Searching the human proteome for analogs of the EEYLKAWTF “mimotope” peptide identified two high-scoring matches (expect values of 283 and 65, respectively), each of 9 residues (EESLKDWFYF and EEYLQAFYF) and therefore representing a potential natural “allotope.” These peptides shared 66% (6/9 identical residues) with the mimotope and possessed the P2E, P9Y/F anchor residues, features of B44-binding peptides. The peptide EESLKDWFYF is derived from an ATPase but is less known about its physiologic role and expression. The peptide EEYLQAFYF is derived from an ATP binding cassette protein ABCD3 involved in transport of fatty acids into the peroxisome.

The ABCD3 Allotope Is an Authentic Alloligand Recognized by LC13

We next examined recognition of the EESLKDWFYF or EEYLQAFYF peptides by LC13.Jurkat cells (CD8+) and LC13 CTL. The EESLKDWFYF peptide did not activate LC13.Jurkat cells and was not examined further because we conclude that this is not a bona fide alloligand for LC13 (not shown). In contrast, both the mimotope and EEYLQAFYF (hereafter allotope) peptides specifically sensitized exogenously loaded T2-B*4405 cells (Figure 2B, middle) and C1R-B*4405 cells expressing ICP47 (Figure 2B, right) for lysis by LC13 CTL.
To determine whether the allotope is naturally presented, the impact of super transfection and knockdown of the ABCD3 gene was studied in cells naturally presenting B*4405-restricted alloantigen to LC13. Super transfection of the ABCD3 gene into C1R.B*4405 cells resulted in a modest increase in constitutive activation of LC13.Jurkat by C1R.B*4405 and C1R.B*4402 cells (Figure S1 available online). A specific RNAi construct was also used to knock down the natural, endogenous expression of ABCD3 in Ag-presenting cells (Figure 2C). Real-time PCR assays of RNA expression showed that the ABCD3 allotope RNAi reduced mRNA expression by >80% and confirmed the specificity of the RNAi constructs (semiquantitative RT-PCR inset, Figure 2C and Figure S2). Mock treatment of cells with irrelevant mβ-actin RNAi had no impact on LC13 allorecognition (Figure 2C, middle panel histograms). In contrast, introduction of the ABCD3 RNAi into the C1R.B*4405 cells specifically reduced constitutive activation of LC13.Jurkat T cells by nearly 50% (p < 0.01) (Figure 2C, right panel histograms). Addition of exogenous allotope to the knocked down Ag-presenting cells restored full activation of the LC13.Jurkat T cells (Figure 2C). These data indicate that the ABCD3 allotope is an authentic, natural alloantigen recognized by the LC13 TCR.

Molecular Mimicry Underpins LC13 Alloreactivity
To understand the structural basis of the LC13 TCR alloreactivity, we determined the structures of the LC13 TCR in complex with the HLA-B*4405 allotope and mimotope complexes to 2.6 Å and 2.7 Å resolution, respectively (Table 1, Tables S1 and S2). These structures were compared with the LC13-virotope complex (Kjer-Nielsen et al., 2003) (rmsd between the allotope and mimotope complex versus the virotope complex was 0.87 Å and 0.77 Å, respectively). This
similarity is reflected in the close superposition of the LC13 TCR in these complexes and their identical 60° docking modes across the long axis of the HLA (Figure 3D). Accordingly, the LC13 TCR location over the C terminus of the HLA-B*4405 antigen-binding cleft mimicked the C-terminal docking of the LC13 TCR on the HLA-B*0801-virotope complex (Kjer-Nielsen et al., 2002a, 2003). The total buried surface area (BSA) at the allotope, mimotope, and virotope interfaces was all \( \approx 2300 \text{ Å}^2 \) and moreover, the shape complementarity at the virotope, allotope, and mimotope interfaces with LC13 was very similar (0.59, 0.64, and 0.60, respectively).

Both the Vα and Vβ domains of the LC13 TCR contributed roughly equally to the interfaces of the allotope, mimotope, and virotope complexes (range: Vα, 51.4%–56.2%, Vβ, 43.8%–48.6%), indicating that the LC13 alloreactivity is not driven by a skewed usage of the V domains at the TCR-pMHC interface unlike other alloreactive complexes (Colf et al., 2007; Reiser et al., 2000). Indeed, the number and nature of the LC13 TCR interactions with the pHLA B*4405 in the allotope and mimotope complexes were also very similar to those of the LC13 TCR-virotope complex (allotope-mimotope-virotope: 146-160-135 van der Waals [v.d.w.] interactions, 15-13-14 H bonds, and 1 salt bridge each; Table S2). Accordingly, the LC13 TCR adopted a strikingly similar footprint on the allogeneic HLA-B*4405-allotope, HLA-B*4405-mimotope, and cognate HLA-B*0801-virotope complex.

**Mimicry of the TCR Footprints and Specific Interactions**

Although the overall docking modes between the LC13 TCR-allotope, mimotope, and virotope complexes were very similar, this does not confirm molecular mimicry at the molecular level. Therefore, we analyzed the individual contacts of the LC13 TCR with each of these three complexes. The relative contact footprints of the complementarity determining region (CDR) loops at the LC13 TCR-pHLA interfaces were also very similar (Figure 3, bottom). Hence, to varying extents, all the CDR loops of the LC13 TCR contributed to virotope, allotope, and mimotope interactions, with only modest differences between them.
T Cell Alloreactivity Mediated by Molecular Mimicry

The CDR2\(\alpha\) loop of the LC13 TCR contributed equally to the interface in the allotope, mimotope, and virotope interactions (approximately 8%–9%; Figures 3A–3C) and interacted via His48\(\alpha\), Leu50\(\alpha\), Ser52\(\alpha\), and Val55\(\alpha\) with the \(\alpha2\) helix of HLA-B*0801 and HLA-B*4405, nesting against the long side chains of Arg151 and Glu154 (Figure 4B). These conserved interactions are mediated predominantly via vdw interactions (Figure 4B; Table S2).

The CDR1\(\beta\) loop minimally participated in the pHLA interactions (Figure 3: Table S2). In contrast, the CDR2\(\beta\) loop contributed equally to the interface in the allotope, virotope, and mimotope interactions (approximately 13%–14%, Figure 4C), through conserved contacts via Tyr48\(\beta\), Gin50\(\beta\), Asn51\(\beta\), Glu52\(\beta\), and Leu55\(\beta\) and the \(\alpha1\) helix of HLA-B*0801 (residues 72–79) and HLA-B*4405 (residues 72–83). This network of polar-mediated contacts includes one conserved salt bridge between Glu52\(\beta\) and Arg79 (Figure 4C). Ala53\(\beta\) makes an additional contact with Arg75 of HLA-B*4405. Interestingly, the CDR2\(\beta\) loop interacted with HLA-B*4405 position 83, a polymorphic site between HLA-B*4405 (Arg83) and HLA-B*0801 (Gly83) (Figure 4C). However, previous mutagenesis has indicated that the CDR2\(\beta\) loop plays a minor energetic role in the LC13 TCR-HLA-B8-virotope interaction and is therefore unlikely to be important in allelo-genic recognition (Borg et al., 2005).

The CDR3\(\alpha\) and CDR3\(\beta\) regions contributed approximately equally at the allotope, virotope, and mimotope interfaces (18.9%–21.4% and 24%–25.6%, respectively; Figure 3). The CDR3\(\beta\) loop dominated contacts with the respective peptides (discussed below), whereas the CDR3\(\alpha\) loop played a larger role in interacting with the HLA heavy chain \(\alpha1\) helix and also forming interactions with Leu94\(\alpha\) and Gin155 of the \(\alpha2\) helix that are conserved across all three complexes (Figure 4D). The conserved interactions between the three complexes also included Gly96\(\alpha\) to the aliphatic base of Arg62; Gly97\(\alpha\) to Ile66; and contacts via Thr98\(\alpha\) and Tyr100\(\alpha\) (Figure 4D). Ser99\(\alpha\) makes a new B*4405 contact not present in HLA B*0801. The CDR3\(\beta\) loop, which abutted the CDR3\(\alpha\) loop and sits centrally above the Ag-binding cleft, mediated contacts with the \(\alpha1\) and the \(\alpha2\) helix of the HLA, in which Gin98\(\beta\) and Tyr100\(\beta\) protruded into the cleft to form conserved interactions along with Leu96\(\beta\) and Gly97\(\beta\).

Accordingly, a very high degree of mimicry of the cognate HLA-B*0801-virotope underpinned LC13 TCR interactions conserved across the HLA-B*4405-allotope and mimotope complexes.

### Peptide-Dependent Molecular Mimicry

Given the differences in the sequences between the cognate virotope, allotope, and mimotope, it was unclear, a priori, whether the peptide-mediated interactions made by the LC13 TCR would be similar between all three complexes. Therefore, we compared the mode of binding of the LC13 TCR to the different peptides.

Upon superposition, the rmsd of the HLA-bound cognate peptide with respect to the bound allotope and mimotope was 0.79 Å and, thus, within the ternary complexes, the peptides adopted similar conformations within the respective Ag-binding cleft (Figure 5). Although the LC13 TCR also interacted with the N-terminal region of the allotope and mimotope peptides (Figures 5A and 5B), the extensive interactions with the

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**Table 1. Data Collection and Refinement Statistics**

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<tr>
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<tr>
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<td>50-2.60 (2.69-2.60)</td>
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<tr>
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<td>3.2 (3.0)</td>
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<tr>
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<td>(R_{merge}) (%)</td>
<td>5.9 (25.6)</td>
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</table>

**Refrinement Statistics**

- Nonhydrogen atoms
  - Protein: 6,657, 13,316
  - Water: 27, 124
  - Resolution (Å): 2.70, 2.60
  - \(R_{factor}\) (%) 19.7, 22.1
  - \(R_{free}\) (%) 26.9, 27.8

- Rms deviations from ideality:
  - Bond lengths (Å): 0.009, 0.006
  - Bond angles (\(^\circ\)): 1.202, 0.926

- Ramachandran plot (%):
  - Most favored region: 87.1, 90.5
  - Allowed region: 12.0, 9.0
  - Generously allowed region: 0.6, 0.4

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The rmsd of the respective CDR2\(\alpha\), CDR3\(\alpha\), CDR1-3\(\beta\) loops within the complexes was <0.45 Å (Table S2). One slight difference was the positioning of the CDR1\(\alpha\) loop between the allotope and virotope complexes (rmsd approximately 1.0 Å) (not shown). Overall, the conformational changes of the LC13 TCR in forming interactions with the virotope complex (Kjer-Nielsen et al., 2002b, 2003) are mirrored in the interactions with the HLA-B*4405-allotope and mimotope structures, despite the differences in the antigenic peptide sequences.

The CDR1\(\alpha\) makes conserved contacts via Gly29\(\alpha\), Thr30\(\alpha\), and Tyr31\(\alpha\) with the \(\alpha2\) helix of HLA-B*4405. The Arg62 of HLA-B*4405 contacts the P1 residue of the allotope, and Tyr159 of HLA-B*4405 contacts Thr30\(\alpha\) (Figure 4A). Thr30\(\alpha\) and Tyr31\(\alpha\) enveloped the “gatekeeper” residue Gin155, which changed conformation upon LC13 TCR ligation in all three complexes. Accordingly, the CDR1\(\alpha\) loop played a similar role in the overall contribution to interactions in the virotope complex (18.2%) when compared to the allotope and mimotope complexes (16%).

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**Notes:**

- Values in parentheses are for highest-resolution shell.
- \(R_{merge} = \sum |I_{hk} - <I_{hk}>| / \sum I_{hk}.
- \(R_{factor} = \sum |F_{o} - |F_{c}| | / \sum |F_{o}| \) for all data except \(R_{free}\).
C-terminal (P6–8) region of the peptides, known to be critical for recognition of the virotope (Kjer-Nielsen et al., 2003), were highly similar in all three complexes. Namely, the C-terminal residues of both the allotope (P6-Ala, P7-Phe, P8-Thr) and mimotope (P6-Ala, P7-Trp, P8-Thr) include a bulky aromatic side chain at P7 that was flanked by small amino acids, a feature important for LC13 recognition of the virotope (P6-Ala, P7-Tyr, P8-Gly) (Figures 5A–5C; Kjer-Nielsen et al., 2003). Consequently, mimicry in this region underpins how the LC13 TCR interacted with the P6–P8 region of the allotope and the mimotope peptides. The small P6 and P8 residues enabled the P7-aromatic to protrude within a central pocket of the LC13 TCR, as well as contributing to specificity-governing interactions with the LC13 TCR. Namely, the P6-Ala made a conserved interaction with Leu94α of CDR3α and Ala99β of CDR3β, and the backbone of P6-Ala formed a conserved H bond with Gln98β of the CDR3β loop (Figures 5A–5C). Despite the different P8 side chains between the virotope (P8-Gly) and allotope/mimotope (P8-Thr), Tyr100β of the CDR3β loop formed a conserved H bond with the backbone of P8 (Figures 5A–5C). The aromatic structures of the P7 residues were each sandwiched between Tyr31α and Tyr100β and contacted Ala99β (Figures 5A–5C). The P7-TyrOH of the virotope formed critical water-mediated interactions with His33α and His48α (Figure 5C); however, because of the differences at this position in the mimotope (P7-Trp) and allotope (P7-Phe), these water-mediated interactions were absent in these complexes (Figures 5A and 5B). The P7-Trp of the mimotope formed a H-bond with Tyr31α (Figure 5B). Interestingly, mutating P7-Phe to P7-Tyr of the allotope increased recognition by the LC13 TCR to levels comparable to that of the cognate interaction (data not shown).

These findings also underscore the lack of recognition of the EESLKDGYF candidate peptide identified in the BLASTp search, because the bulky side chain of P8-Tyr in this ligand...
would sterically obstruct recognition of the P7 aromatic crucial to recognition of the B*4405-virotope, allotope, and mimotope complexes. Accordingly, in addition to the mimicry between the surface topology of the HLA-B*0801 and B*4405 heavy chains, substantial mimicry of the HLA-B*0801-restricted virotope underscored how the LC13 TCR interacted with the critical C-terminal region of the HLA-B*4405-restricted allotope and mimotope.

**Alloreactivity Discriminates between Related B44 Allotypes**

LC13 alloreacts with HLA-B*4402 and HLA-B*4405, but surprisingly not with HLA-B*4403 (Burrows et al., 1994, 1995, 1997). Therefore, we tested LC13 recognition of phytohaemagglutinin (PHA) blast cells expressing either HLA-B*4405, HLA-B*4402, or HLA-B*4403 after adding exogenous mimotope or allotope peptide (Figure 6A). Consistent with the defined specificity of LC13 (Burrows et al., 1997), the HLA-B*4403+ cells were not recognized at physiological concentrations of the mimotope peptide. This might partly reflect lower binding of the allotope peptide to B*4403 (not shown). Interestingly, the HLA-B*4405+ cells presented both peptides more efficiently than did HLA-B*4402. Notably, the allotope and mimotope peptides complexed with HLA-B*4405 were recognized at even lower peptide concentrations than the cognate FLRGRAYGL virotope peptide, presented by HLA-B*0801+ PHA blasts (Figure 6A). This difference appeared to result from differential T cell recognition of these ligands rather than differences in peptide-HLA binding affinity, as shown by the fact that cross-blocking of pHLA-tetramer staining of LC13-like T cells confirmed the binding hierarchy HLA-B*4405-mimotope > HLA-B*4405-allotope > HLA-B*0801-virotope tetramer (Figure S3).

We then tested whether fine specificity of alloreactivity and pHLA-tetramer staining correlated with the affinity of the LC13 TCR-pHLA interaction via surface plasmon resonance (SPR) studies. The LC13 TCR bound to the HLA-B*4405-mimotope complex with comparable affinity to the HLA-B*4402-mimotope complex (Kd = 1.5 μM and 1.3 μM, respectively) but interacted...
Figure 5. Mimicry in Peptide-TCR Contacts

Contacts between the LC13 TCR and the allotope EEYLQAFTY (dark blue) (A), the mimotope EEYLKAWTF (orange) (B), and the virotope FLRGRAYGL (purple) (C). The peptide is represented in stick format and the LC13 TCR side chains involved in peptide contact are shown. Colors: CDR1\(\alpha\), red; CDR3\(\alpha\), blue; and CDR3\(\beta\), cyan. The LC13 TCR makes conserved contacts with the allotope (A), mimotope (B), and virotope (C) at positions P6–P8. In addition, the LC13 TCR makes some water-mediated contacts (red dash lines) via His33\(\alpha\) and His48\(\alpha\) with the Tyr7 of the virotope (C). The interactions made by the LC13 TCR with P4-Leu of both the allotope and mimotope peptides were exclusively via residues from the CDR3\(\alpha\) loop and collectively this resulted in a greater contribution of the CDR3\(\alpha\) loop in contacting the mimotope (48.5%) and allotope (47%) when compared to the CDR3\(\alpha\)-mediated contacts of the virotope (37%). The CDR1\(\alpha\) loop of the LC13 TCR contacts P3 of the mimotope.

The conformation of the allotope and mimotope in their respective LC13-ternary complexes were very similar (rmsd = 0.31 Å) (Figure 6D). However, when the structures of the HLA-B*4405-allotope and HLA-B*4405-mimotope were compared in the absence of LC13 TCR ligation, the conformation of the allotope and mimotope differed markedly (rmsd 0.94 Å) whereas the HLA Ag-binding cleft adopted the same conformation (rmsd 0.20 Å) (Figure 6E). Namely, there were major differences in the conformation of the peptides between P3-Tyr and P7-Trp/Phe, where, for example, P5-Gln of the allotope and the P5-Lys of the mimotope pointed down or upwards from the Ag-binding cleft, respectively.

This observation revealed that conformational plasticity of the allotope and mimotope play an important role in the alloresponse (compare Figures 6D and 6E). For example, upon ligation, the P3-Tyr of the allotope rotated downwards to avoid steric clashes with Gln155, but nevertheless maintained an H bond with Asp156 and formed an additional H bond to Asp114 (Figure 6F). Additionally, the mimotope is significantly remodeled upon LC13 TCR ligation (Figure 6G). Namely, the CDR1\(\alpha\) and CDR3\(\alpha\) loops pushed down the central region of the mimotope (Figure 5B), causing P4-Leu to be shifted aside and P5-Lys to flip downwards into the Ag-binding cleft, forming an H bond to Tyr116 and salt bridging to Asp114 and Asp156 (Figure 6F). The movement of Gln155 (Figure 4D) also caused a remodeling of P3-Tyr and P7-TRp, where the P3-Tyr rotated downwards to form a H bond with Asp156 and the P7-Trp side chain flips 180° to establish more contacts with the LC13 TCR. Collectively, the mimotope and allotope mimicked the conformation of the virotope only in the ligated state and thus peptide-dependent molecular mimicry is “forced” by the LC13 TCR (Figures 6D and 6E). However, the LC13 TCR-induced plasticity of the mimotope and allotope would be disfavored in HLA-B*4403 as a result of Leu156. Namely, similar plasticity of the mimotope would result in a buried and uncompensated charge at P5-Lys. Regarding the
HLA-B*4403-allotope complex, movement of P3-Tyr would result in its hydroxyl moiety being unfavorably located in a hydrophobic pocket. Thus, the fine specificity of the alloreactivity was partly a consequence of the differential ability of HLA-B*4405, HLA-B*4402, and HLA-B*4403 to accommodate plasticity of the mimotope and allotope upon TCR ligation, further highlighting the role and sensitivity of peptide-dependent molecular mimicry.

**DISCUSSION**

Molecular mimicry, namely when similar structures from dissimilar proteins function in similar ways, is considered to underpin receptor-ligand cross-reactivity in many biological systems (Mariuzza and Poljak, 1993; Oldstone, 1987) and represents a central tenet for therapeutic development of analog drugs. Described in the 1980s in an immunological context (Williams, 1983), molecular mimicry is thought to be the basis for a number of B cell autoimmune disorders, whereby the epitope from the pathogen mimics the conformation of the self-ligand (Oldstone, 1987; Rose and Mackay, 2000). Evidence for molecular mimicry of T cell ligands, though long suspected, has been harder to establish structurally (Quaratino et al., 1995) because of the dual specificity of T cell recognition for MHC and peptide. Nonetheless, evidence is accumulating for mimicry as a basis of some T cell autoimmunity (Harkiolaki et al., 2009; Hausmann et al., 1999; Wucherpfennig and Strominger, 1995) and that T cell cross-reactivity may be dependent on a few conserved germ-line-encoded interactions (Dai et al., 2008). Here we describe how extensive molecular mimicry underpins direct, human T cell alloreactivity, a structurally unresolved phenomenon that leads to tissue destruction and transplant rejection. In antiviral immunity, small differences in the peptide or HLA molecule can

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**Figure 6. Fine Specificity of the Alloreaction**

(A) LC13 recognizes the mimotope and allotope peptides presented by HLA-B*4402 and HLA-B*4405 but not HLA-B*4403. LC13 CTL cytotoxicity of PHA-stimulated T cell lines expressing either HLA-B*4405, HLA-B*4402, or HLA-B*4403. PHA blasts downregulate MHC-I and lose their capacity to be lysed by the LC13 CTLs allowing them to be used as exogenous peptide-presenting targets. Dose response of cytotoxicity on allotope (triangles) or mimotope (circles) peptide. Lysis of HLA-B*0801-positive PHA blasts loaded with the virotope (squares) is also shown.

(B and C) LC13 TCR binding of HLA-B*4405, HLA-B*4402, and HLA-B*4403 in complex with the mimotope (B) and the allotope (C) as determined by SPR.

(D) Superposition of the allotope (blue-green) and the mimotope (orange) bound to the HLA-B*4405 in the LC13 TCR ligated state.

(E) Structure of the allotope (pink) superposed on the mimotope (green) in complex with HLA-B*4405 but unliganded by LC13.

(F) Conformational change of the allotope in the nonligated (pink) and LC13 TCR-ligated state (marine).

(G) Conformational change of the allotope in complex with HLA-B*4405 both unliganded (green) and liganded (orange) to the LC13 TCR. During the LC13 TCR ligation, the mimotope undergoes a structural change with the flipping of Lys5. The polymorphic HLA positions (Tyr116 and Asp156 in HLA-B*4405) and the conserved Asp114 are shown in stick format.
effectively ablate TCR corecognition of the viral determinant bound to the HLA molecule. Thus, a priori it was unexpected that a human T cell alloreactivity between disparate HLA allotypes was attributable to molecular mimicry. Indeed, a previously described example of murine T cell alloreactivity showed how the TCR adopted markedly different docking strategies when recognizing self versus foreign ligands (Colf et al., 2007). To exemplify this point further, HLA-B*4405 differs from HLA-B*0801 by 25 amino acids in the Ag-binding cleft, of which 5 residues (positions 80, 82, 83, 163, and 167) are surface exposed and potentially available for TCR contact. In the 2C TCR system of alloreactivity, H2-K\textsuperscript{d} and H2-L\textsuperscript{d} molecules differ by 31 residues, of which only 4 polymorphic residues are solvent exposed for potential TCR contact (Colf et al., 2007). Moreover, if one considers the apparent relatedness between the surface topologies of HLA-B8 and HLA-B44, one would have anticipated that the LC13 TCR alloreacts against all HLA-B44 allotypes, and this is clearly not the case for HLA-B*4403, which differs from HLA-B*4405 by only two buried polymorphic residues (Zernich et al., 2004).

In our study, the viral and alloptope peptides adopted similar conformations only after binding the TCR. This induced-fit mechanism of molecular mimicry further explained why the TCR could effectively discriminate between subtle polymorphic differences between the foreign HLA-B*4402, HLA-B*4405, and HLA-B*4403 allotypes. Thus, our data not only highlight the intricate peptide dependence of T cell alloreactivity but also show that direct T cell alloreactivity is attributable to exquisite specificity of the TCR rather than degenerate recognition of MHC. Our findings suggest that in transplantation, nonpermissive taboo mismatches (Doxiadis et al., 1996) might depend on serendipitous mimicry that is lacking in permissive mismatches.

Our observations in the LC13 TCR system and the contrasting observations in the 2C TCR system raise the intriguing question of whether molecular mimicry, or alternatively, disparate docking modes between the cognate and allo-ligand will best explain the general phenomenon of alloreactivity. The LC13 TCR system describes alloreactivity between two disparate allotypes. Because the LC13 TCR can alloreact via mimicry between these two disparate allotypes, then it follows that alloreactivity between more related alleles is likely to arise from mimicry. Moreover, it also anticipated that molecular mimicry operates between the alloreactions between HLA-B8 and HLA-B*3508 (Archbold et al., 2006) and between HLA-B*3508 and HLA-B44 (Tyan et al., 2005). Moreover, as T cells undergo thymic selection and multimeric LC13.

Identification of Endogenous Alloligand

A randomized peptide library was engineered in complex with HLA-B*4405 molecules in a baculovirus vector (Crawford et al., 2006). The potential nongenic “allopeptide” library was constructed with random oligornucleotides but fixing codons encoding P1E, a residue not likely to be involved in LC13 recognition and the HLA-B*4405 anchor sites P2E and P9F or Y. PCR fragments encoding the library were ligated to constructs encoding \textit{b}2microglobulin and the HLA-B*4405 heavy chain directing expression of individual HLA-B*4405-peptide complexes from each virus. Sf9 cells were infected with the amplified viral stocks containing the HLA-B*4405-peptide library so that each infected cell displayed a unique peptide-HLA complex. Cells were costained with fluorocinated anti-\textit{b}2microglobulin, and fluorcrome-labeled LC13 TCR ectodomain made mutemeric with an anti-TCR mAb. Rare cells expressing HLA-B*4405-peptide complexes that bound LC13 TCR were repeatedly sorted and expanded by culture in vitro. After the 4th sorting, SF9 cells homogeneously expressed a ligand that bound both anti-\textit{b}2microglobulin and mutemeric LC13.

T Cell Activation Assays

CTL killing assays (Burrows et al., 1994) and activation of Jurkat.LC13 cells were assayed as previously described (Beddoe et al., 2009). Essentially, Jurkat.LC13 cells (10\textsuperscript{5}) were cocultured with 10\textsuperscript{5} antigen-presenting cells for 4 hr at 37°C in the absence or presence of peptide. Expression of CD69 was
then detected by flow cytometry gating on GFP-positive LC19.Jurkat cells. T cell activation was measured as the percentage of CD69-positive cells among the GFP-positive LC19.Jurkat cells relative to the unstimulated population. RNAi knockdown of ABCD3 is described in the Supplemental Data. Primary T cells were obtained from blood donors with the approval of the Australian Bone Marrow Donor Registry Ethics Committee Scientific Review Panel.

Additional Data
Supplemental Data include protein expression, purification, crystallization, structure determination, and SPR measurement.

ACCESSION NUMBERS
Coordinates have been deposited in the PDB (codes: 3KPL, 3KPM, 3KPQ, 3KPP, 3KPS, 3KPR).

SUPPLEMENTAL DATA
Supplemental Data include Supplemental Experimental Procedures, seven figures, and three tables and can be found with this article online at http://www.cell.com/immunity/supplemental/S1074-7613(09)00510-X.

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REFERENCES


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