

# The structure of the human allo-ligand HLA-B\*3501 in complex with a cytochrome p450 peptide: Steric hindrance influences TCR allo-recognition

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Virus-specific T cell populations have been implicated in allo-recognition. The subdominant T cell receptor JL12 recognizes both HLA-B\*0801 presenting the Epstein–Barr virus-derived peptide FLRGRAYGL and also HLA-B\*3501 presenting the cytochrome p450 self peptide KPIVVLHGY. This cross-reactivity could promote the rejection of HLA-B\*3501-positive cells in Epstein–Barr virus-exposed HLA-B\*0801 recipients. LC13, the dominant TCR against the HLA-B\*0801:FLRGRAYGL complex, fails to recognize HLA-B\*3501:KPIVVLHGY. We report the 1.75-Ångstrom resolution crystal structure of the human allo-ligand HLA-B\*3501:KPIVVLHGY. Similarities between this structure and that of HLA-B\*0801:FLRGRAYGL may facilitate cross-recognition by JL12. Moreover, the elevated peptide position in HLA-B\*3501:KPIVVLHGY would provide steric hindrance to LC13, preventing it from interacting in the manner in which it interacts with HLA-B\*0801:FLRGRAYGL. These findings are relevant to understanding the basis of T cell cross-reactivity in allo-recognition, optimal transplant donor-recipient matching and developing specific molecular inhibitors of allo-recognition.

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## Introduction

The role of cytotoxic T lymphocytes (CTL) in allograft rejection is well recognized and HLA-B mismatches influence transplant survival more than HLA-A mismatches [1, 2]. Most T cell responses in early allograft rejection result from direct recognition of self peptides presented by allo-MHC molecules [3]. Two mechanisms,

which may co-exist, could account for the high frequency of such allo-reactive T cells. Firstly, T cell receptors (TCR) could recognize new determinants on the surface of the allo-MHC. Secondly, TCR may recognize self peptide epitopes, which are not presented by host MHC molecules or are presented in a different structural conformation. An individual's immune history might influence their allo-response if self MHC-restricted virus-specific T cells cross-react with structurally similar complexes of self peptide and allo-MHC [4–6].

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**Abbreviation:** **rmsd:** root mean square deviation

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T cell cross-reactivity has been reported between the HLA-B\*0801-restricted EBV EBNA-3-derived latent cycle peptide FLRGRAYGL and an HLA-B\*3501 allo-antigen [7]. HLA-B\*0801 individuals typically use an essentially similar (immunodominant) TCR, the LC13 receptor, to recognize this viral peptide [8]. However, individuals who express both HLA-B\*0801 and HLA-B\*4402 do not respond to HLA-B\*0801:FLRGRAYGL with the usual dominant TCR and instead use a diverse array of subdominant TCR [9]. These subdominant clones display allo-reactivity and one clone with receptor JL12 recognizes HLA-B\*3501 presenting the self peptide KPIVVLHGY found in human cytochrome p450 [7]. LC13 does not recognize HLA-B\*3501. We have solved the structure of the human allo-ligand HLA-B\*3501:KPIVVLHGY and modeled the receptors onto it to explore why JL12 cross-reacts with this pMHC, but LC13 does not.

## Results

### Overview of the HLA-B\*3501:KPIVVLHGY structure

The structure of HLA-B\*3501 in complex with the cytochrome p450-derived self peptide KPIVVLHGY was determined to 1.75-Å resolution (Table 1; Fig. 1A). The C $\alpha$  backbone of the  $\alpha$ 1/ $\alpha$ 2 domain from residues 3–175 of HLA-B\*3501:KPIVVLHGY is superimposed on the equivalent region of HLA-B\*0801:FLRGRAYGL [10] with a root mean square deviation (rmsd) of 0.85 Å (Fig. 1B). There is close superposition between the TCR-accessible surfaces of the two MHC molecules in all but the short portion of the  $\alpha$ 2 helix (residues 141–152), a characteristic difference of HLA-B\*B35 alleles compared to other pMHC structures as previously noted [11].

### Features of the peptide conformation relevant to allo-recognition

The central regions of the peptides differ substantially (Fig. 1C), partly due to the different anchor pocket positions at P2 and the terminal PC in HLA-B35, and P3, P5 and PC in HLA-B8 [12, 13]. Consequently, at peptide positions P4 and P5, the KPIVVLHGY main chain is some 2 Å higher above the peptide binding groove than the equivalent region of FLRGRAYGL. The bulky surface-exposed valine side chain at P4 (compared to a glycine in FLRGRAYGL) further accentuates this difference. Relative to KPIVVLHGY, the FLRGRAYGL peptide has a slightly elevated main chain kink at peptide position P6. Both peptides have an exposed cyclic side chain at position P7, a histidine in KPIVVLHGY and a tyrosine in FLRGRAYGL. In the LC13 TCR-HLA-B\*0801:

FLRGRAYGL structure [14], the P7 tyrosine was accommodated in an 11×11 Å pocket between the CDR3 $\alpha$  and CDR3 $\beta$  loops. This pocket could potentially accommodate the histidine in the HLA-B\*3501-KPIVVLHGY structure.

### Features of the MHC protein relevant to allo-recognition

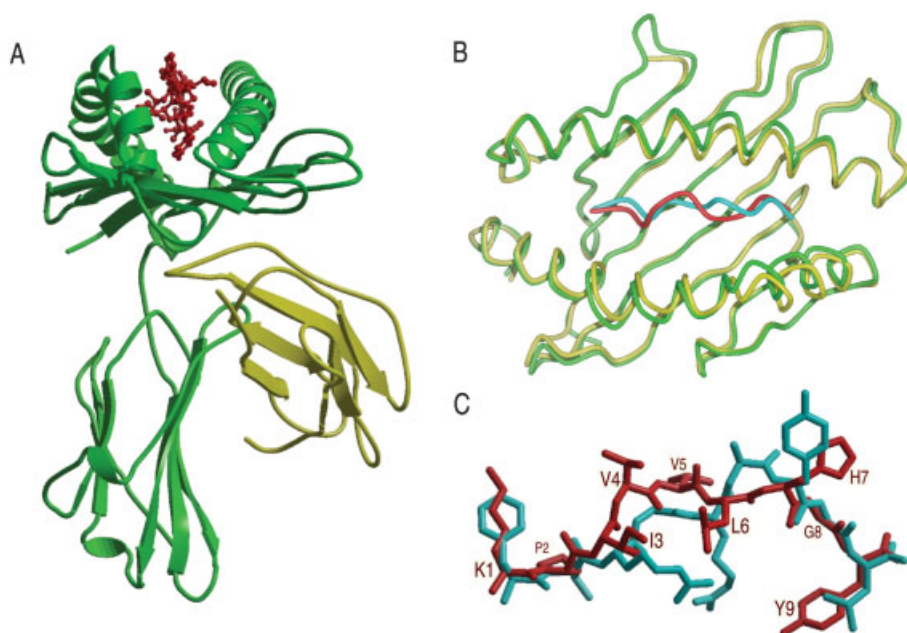
Allo-reactivity could reflect recognition of features on the allo-MHC that are absent on the self MHC molecule. Of the 19 differences between mature HLA-B\*0801 and HLA-B\*3501, only two in HLA-B\*3501, at positions 156 and 163, are potentially upward-facing and TCR-

**Table 1.** Data processing and refinement statistics

Resolution	1.75 Å
Space group	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
Unit cell	a=51.32 Å b=82.25 Å c=109.85 Å
R <sub>merge</sub> <sup>a)</sup>	7.2% (58.4%)
I/ $\sigma$	23.9 (4.0)
Number of unique reflections	47 620
Redundancy	7.8
Completeness	100%
R <sub>cryst</sub> <sup>b)</sup>	19.7% (22.5%)
R <sub>free</sub>	22.7% (24.4%)
Model atoms	
Non-hydrogen protein atoms	3158
Water molecules	325
Heterogen molecules (glycerol)	18
Average temperature factors	
Protein main chain atoms	18.3 Å <sup>2</sup>
Protein side chain atoms	21.3 Å <sup>2</sup>
Ordered solvent molecules	29.2 Å <sup>2</sup>
rmsd from ideal geometry	
Bond distances	0.011 Å
Bond angles	1.27°
Ramachandran plot	
Most favored region	92.2%

<sup>a)</sup> R<sub>merge</sub> indicates  $\sum_{hkl} \sum_i |I_i - \langle I \rangle| / \langle I \rangle$  where  $I_i$  is the intensity for the  $i^{\text{th}}$  measurement of an equivalent reflection, with indices  $h$ ,  $k$ , and  $l$ .

<sup>b)</sup> R<sub>cryst</sub> indicates  $(\sum ||F_o| - |F_c|| / \sum |F_o|)$  using all data except 4%, which were used for the R<sub>free</sub> calculation. Values for the highest resolution shell are indicated in parentheses.



**Figure 1.** The structure of HLA-B\*3501:KPIVVLHGY. (A) The HLA-B\*3501:KPIVVLHGY structure. The MHC heavy chain is shown in green,  $\beta$ 2 microglobulin in yellow and the peptide in red ball and stick representation. (B) Superposition of the  $\alpha$ 1/ $\alpha$ 2 domains of HLA-B\*3501:KPIVVLHGY (green) with HLA-B\*0801:FLRGRAYGL (yellow). The portion of the  $\alpha$ 2 helix that differs between the structures is at the bottom right of the figure. (C) Comparison of the KPIVVLHGY (red) and FLRGRAYGL (blue) peptides as presented by HLA-B\*3501 and HLA-B\*0801, respectively. The peptide backbone is relatively elevated around peptide positions P4 and P5 in the HLA-B\*3501:KPIVVLHGY structure.

accessible (Fig. 2A). An exposed aspartate at position 156 in HLA-B\*0801 is replaced by leucine in HLA-B\*3501, which points inwards towards the binding groove and is not accessible to the TCR. A threonine at position 163 in HLA-B\*0801 is replaced by a leucine in HLA-B\*3501, which is exposed for potential interaction with TCR. The structure of HLA-B\*0801:FLRGRAYGL bound to the LC13 TCR [14] was superimposed (rmsd of 1.24 Å) onto residues 3–175 of HLA-B\*3501:KPIVVLHGY (Fig. 2B). This demonstrated that position 163 of HLA-B\*3501 molecule would not contact the TCR bound in this orientation. Thus, if JL12 binds with a similar orientation to LC13, determinants on the allo-MHC will not account for allo-recognition of HLA-B\*3501:KPIVVLHGY.

### Steric hindrance of the CDR3 $\alpha$ loop and allo-recognition

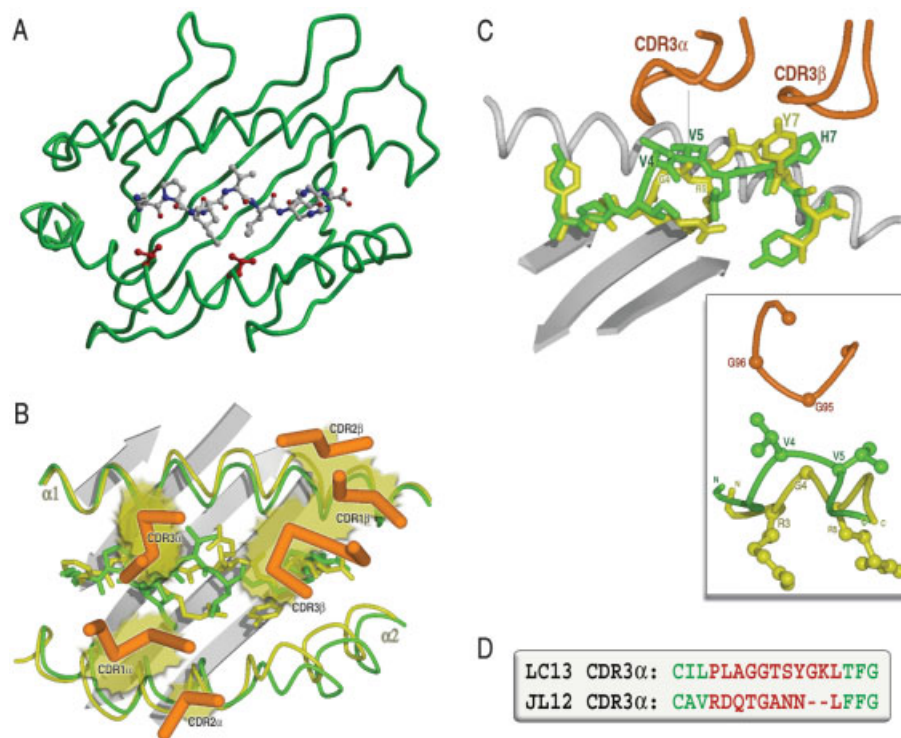
Upon binding HLA-B\*0801:FLRGRAYGL, the LC13 CDR3 $\alpha$  loop loses its canonical conformation, undergoing a large 8-Å shift away from the peptide to make extensive contacts with the MHC  $\alpha$ 1 domain [14]. LC13 tolerates only alanine, glycine or proline at position P4 in the HLA-B\*0801:FLRGRAYGL complex [9]. However, the elevated peptide backbone and protruding P4 valine in the HLA-B\*3501 complex would provide steric hindrance to the LC13 CDR3 $\alpha$  loop, preventing LC13

from binding HLA-B\*3501:KPIVVLHGY in the same orientation that it binds HLA-B\*0801:FLRGRAYGL (Fig. 2B, C). Prominent differences between peptide sequences and conformations thus result in steric hindrance which accounts for the inability of the LC13 TCR to recognize the allo-ligand HLA-B\*3501:KPIVVLHGY in the way that it recognizes HLA-B\*0801:FLRGRAYGL.

### Binding of the JL12 TCR

The full-length sequence of the TCR of the CTL clone JL12 was determined. The TCR $\alpha$  and TCR $\beta$  sequences were consistent with the V-D-J usage described for clones JL7/12/20 and RL10/45 (AV7S4, RDQTGANNL, AJ17S5 and BV7S5, SHGTSGILETQ, BJ2S5) [7]. The JL12 and LC13 TCR sequences were aligned (Fig. 2D) and LC13 residues that contact HLA-B\*0801:FLRGRAYGL identified by examination of the published structure [PDB: 1MI5] [14].

We modeled interactions that would arise if the JL12  $\alpha$  chain binds HLA-B\*0801:FLRGRAYGL with a similar orientation to that adopted by the LC13 TCR (Fig. 2B). Sequence differences between the JL12 and LC13  $\alpha$  chains, at position 30 (Thr to Phe) of CDR1 $\alpha$ , positions 48 (His to Tyr) and 50 (Leu to Val) of CDR2 $\alpha$ , and positions 98 (Thr to Ala) and 100 (Tyr to Asn) of CDR3 $\alpha$ , might be accommodated within a



**Figure 2.** Factors influencing cross-reactivity. (A) Residues that differ between HLA-B\*3501 and HLA-B\*0801. Polymorphic residues that are potentially TCR-accessible in HLA-B\*3501:KPIVVLHGY are shown as red ball and stick representations. Position 156 (right) points into the peptide-binding groove and position 163 (left) lies outside the LC13 contact region [see (B)]. (B) Superposition of HLA-B\*3501:KPIVVLHGY (green) onto HLA-B\*0801:FLRGRAYGL (yellow) in complex with the LC13 TCR (orange loops). The MHC chains have sequence identity in the regions of HLA-B\*0801 that are contacted by LC13. (C) Steric constraints on LC13 binding of HLA-B\*3501 allo-ligand. The superposition is the same as in (B) and the MHC  $\alpha$ 1 helix and other TCR loops are not shown for clarity. Main figure: The KPIVVLHGY peptide (green) is elevated at peptide positions P4 and P5 compared to FLRGRAYGL (yellow). Inset: The raised relative position of KPIVVLHGY (green) at positions P4–P5 would prevent the LC13 CDR3 $\alpha$  loop (orange) from using the binding position adopted for HLA-B\*0801:FLRGRAYGL. (D) Sequences of the CDR3 $\alpha$  region of the LC13 and JL12 TCR  $\alpha$  chains. The JL12 loop is two residues shorter.

broadly similar binding interface, while the substitution at position 52 (Ser to Asp) could allow new hydrogen bond formation balancing that lost by the substitution at position 31 (Tyr to Gly). In contrast, the TCR  $\beta$  chain is unlikely to bind in a structurally conserved manner. The JL12 CDR3 $\alpha$  loop is only nine amino acids long, two residues shorter than that of LC13, and the shorter JL12 loop should be able to interact with the allo-ligand without the steric hindrance encountered by the longer LC13 loop.

## Discussion

The responses to HLA-B\*0801:FLRGRAYGL and HLA-B\*3501:KPIVVLHGY are particularly interesting and potentially informative about TCR recognition and allo-recognition. Polymorphisms between HLA-B\*0801 and HLA-B\*3501 are unlikely to account for their discrimination by LC13 or their cross-recognition by JL12, as only two polymorphic residues are TCR-accessible and

neither is positioned to make TCR contacts in the orientation used by LC13. The central region of the peptide lies higher above the peptide-binding groove in HLA-B\*3501:KPIVVLHGY than in HLA-B\*0801:FLRGRAYGL. There is significant conformational movement of the LC13 CDR3 $\alpha$  loop upon binding to HLA-B\*0801:FLRGRAYGL. However, LC13 could not interact with HLA-B\*3501:KPIVVLHGY in this way, because this conformational change would be prevented by steric hindrance from the elevated peptide position and bulky valine residues in the KPIVVLHGY peptide. Mutation of this LC13 loop strongly diminished the affinity for HLA-B\*0801:FLRGRAYGL, confirming its energetic importance in the interaction [15].

Unlike LC13, the JL12 receptor may bind both HLA-B\*0801 and HLA-B\*3501 in a similar orientation without such structural constraints because its CDR3 $\alpha$  chain is significantly shorter. For the recognition footprint defined by LC13, the only major TCR-accessible differences between the two pMHC complexes are in the peptide. Therefore, to bind both pMHC ligands

with a similar orientation to LC13, the JL12 receptor merely requires sufficient conformational flexibility without steric hindrance from the ligands, to accommodate the different peptide conformations and side chains. This is consistent with allo-recognition being likely when self and allo-MHC have similar scaffolds that both interact with recipient TCR. Further studies of allo-ligands and receptors should establish to what degree this is generally true and the extent to which steric hindrance affects allo-recognition in other contexts.

Crystal structures exist for the murine BM3.3 TCR bound to the allo-ligand H-2K<sup>b</sup> complexed to two different peptides [16]. In one structure, the CDR3 $\alpha$  loop folds away from the peptide to contact the  $\alpha$ 1 helix; in the other, it swings inwards to contact the peptide. If the loop could not make a favorable peptide interaction and was sterically hindered from moving outwards, recognition of potential allo-ligands might not occur. Modeling based on the structure of the 2C TCR bound to the self ligand H-2K<sup>b</sup> and the structure of the allo-ligand H-2L<sup>d</sup> suggests that the allo-ligand peptide bulges towards the TCR  $\beta$  chain [17]. This bulge could produce steric hindrance that would block allo-recognition by TCR with long CDR loops. Thus, murine studies are consistent with a potential role for steric hindrance of TCR loop movement in determining whether TCR interact with allo-ligands.

The outcome of transplantation depends on the number of MHC mismatches and on the actual alleles that are mismatched [18]. Structural comparisons imply that allo-responses involving HLA-B\*0801 and HLA-B\*3501 may be favored by the structural equivalence of the MHC surfaces contributing to TCR recognition and that this may outweigh profound differences in peptide conformation, unless steric hindrance occurs. The immunodominant TCR LC13 uses conformational changes in the CDR3 $\alpha$  loops to bind HLA-B\*0801:FLRGRAYGL in a highly peptide-specific manner. Subdominant TCR, such as JL12, discriminate less stringently between peptides and cross-react with allo-peptides presented by HLA-B\*3501, which conserves the MHC contribution to the TCR-pMHC interface. Recent studies have pointed to certain peptide characteristics favoring immunodominant TCR responses [19]. Information on the balance between MHC and peptide interactions which allow TCR to discriminate between pMHC ligands will further our understanding of allo-recognition. This could aid transplant matching, post-transplant monitoring for rejection and the development of specific therapeutic blockade of allo-responsive T cells.

## Materials and methods

### Protein production and crystallization

Soluble HLA-B\*3501 (residues 1–276) and  $\beta$ 2 microglobulin (residues 1–99) were expressed as inclusion bodies in *Escherichia coli*, refolded with KPIVVLHGY peptide as described previously [20] and refolded complex was purified by gel filtration. Needle-like crystals grew at room temperature by vapor diffusion from sitting drops containing 1  $\mu$ L of 21% polyethylene glycol 4000, 0.14 M ammonium acetate, 0.07 M sodium citrate pH 5.6 and 1  $\mu$ L of 9 mg/mL protein.

### Data collection, processing and structure determination

Crystals were flash-frozen in 15.75% polyethylene glycol 4000, 0.105 M ammonium acetate, 0.0525 M sodium citrate pH 5.6 with 25% glycerol. Diffraction data were collected at 100 K from a single crystal at the European Synchrotron Radiation Facility station ID14-EH3 (Grenoble, France). Data were indexed and scaled using the HKL programs [21]. The structure was solved by molecular replacement using the program AMoRe [22] and the HLA-B\*3501-HIV1 Nef (75–82) structure [PDB entry: 1A1N] without peptide or water molecules as a search model [11]. After rigid body refinement, the KPIVVGHLY peptide was rebuilt into difference electron density maps and the model improved with cycles of positional and B factor refinement and simulated annealing in the CNS programme [23] with manual model building in O [24]. Ordered solvent molecules were added where appropriate and final rounds of refinement completed with REFMAC [25]. Structural superpositions were done using SHP [26]. Figures were prepared using Molscript [27] and Bobscript [28]. The structure was validated and submitted to the PDB database with accession code 2CIK.

### Determination of the JL12 TCR sequence

RNA was extracted from JL12 CTL; single-stranded cDNA was primed with oligo(dT) and poly(G) tails added with terminal transferase (Stratagene). Anchor PCR was performed with *Pfu* polymerase (Stratagene), a poly(C) primer and a reverse primer specific for either the TCR $\alpha$  constant region (GAGA-GAGAGGTACCTGCAGGAACCTTCTGGGCTGGGGAAGAAGG) or the TCR $\beta$  constant region (GAGAGAGAGGTACCTGCAGTCTGCTCTACCCAGGCTCGGC). PCR products were cloned and sequenced.

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