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Research Article

Structural basis of the TCR-pHLA complex provides insights into the unconventional recognition of CDR3β in TCR cross-reactivity and alloreactivity

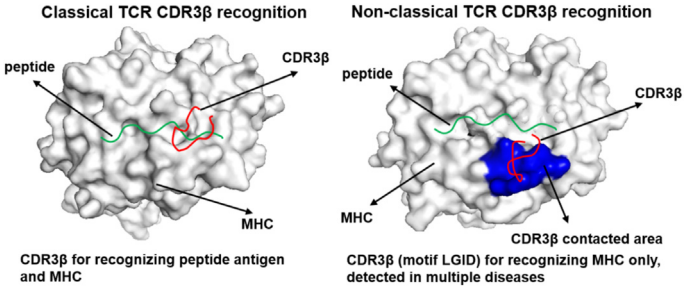
Dan San [1], Jun Lei [1], Yang Liu [1], Baowei Jing, Xiang Ye, Pengcheng Wei, Chonil Paek, Yi Yang, Jin Zhou, Peng Chen, Hongjian Wang, Yongshun Chen [**], Lei Yin [*]

State Key Laboratory of Virology, Hubei Key Laboratory of Cell Homeostasis, College of Life Sciences, Department of Clinical Oncology, Renmin Hospital of Wuhan University, Wuhan University, Wuhan, China

HIGHLIGHTS

- TCR T18A induces a backbone switch of the TL9 peptide in B4201 to approach its conformation in B8101.
- The flexibility of T18A CDR loops enables adaptation to polymorphic HLA ligands.
- CDR3β of T18A TCR shifts to avoid the peptide antigen but intensively recognizes the HLA only.
- Featured sequence pairs of CDR3β and HLA were found in multiple diseases.

GRAPHICAL ABSTRACT



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ABSTRACT

Evidence shows that some class I human leucocyte antigen (HLA) alleles are related to durable HIV controls. The T18A TCR, which has the alloreactivity between HLA-B*42:01 and HLA-B*81:01 and the cross-reactivity with different antigen mutants, can sustain long-term HIV controls. Here the structural basis of the T18A TCR binding to the immunodominant HIV epitope TL9 (TPQDLNTML180-188) presented by HLA-B*42:01 was determined and compared to T18A TCR binding to the TL9 presented by the allo-HLA-B*81:01. For differences between HLA-B*42:01 and HLA-B*81:01, the CDR1α and CDR3α loops adopt a small rearrangement to accommodate them. For different conformations of the TL9 presented by different HLA alleles, not like the conventional recognition of CDR3s to interact with peptide antigens, CDR3β of the T18A TCR shifts to avoid the peptide antigen but intensively recognizes the HLA only, which is different with other conventional TCR structures. Featured sequence pairs of CDR3β and HLA might account for this and were additionally found in multiple other diseases indicating the popularity of the unconventional recognition pattern which would give insights into the control of diseases with epitope mutating such as HIV.

* Corresponding author.
** Corresponding author.
E-mail addresses: yongshun2007@163.com (Y. Chen), yinlei@whu.edu.cn (L. Yin).
[1] These authors contributed equally to this work.

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1. Introduction

Antigen-specific T cell immunity is specified by the interaction of their T cell receptor (TCR) with self-peptide-MHC complexes. In general, mature T cells recognize only foreign antigens presented by MHC molecules after positive and negative selection in thymus(Allen, 1994; Kisielow & von Boehmer, 1991; Marusić-Galesić & Pavelić, 1990; Saito & Watanabe, 1998; Starr et al., 2003). However, previous studies reported that T cells sometimes cross-restricted with both self and non-self HLAs(Galperin & Farenc, 2018; Hawke et al., 1999; Hennecke & Wiley, 2002; Mukhopadhyay et al., 2017; Vollmer et al., 2000; Yousef et al., 2012), mainly involving class II MHCs. In other words, some T cells can break the restriction of HLA and can also react directly with HLA molecules from unrelated individuals(Colf et al., 2007; Felix & Allen, 2007; Sherman & Chattopadhyay, 1993), which is called 'alloreactivity' and can induce extra immune responses(Mehrotra et al., 2015; Nagy, 2012; Sicard et al., 2020). Such alloreactivity is harmful to transplanted cells that patients with some HLA mismatches can have severe T cell immune responses and result in poor results of transplantation, known as taboo mismatches(Doxiadis et al., 1996; Kawase et al., 2007). And many pieces of evidence suggests that T cell cross-restriction is a major cause of tissue transplant-related morbidity and mortality(Fleischhauer et al., 1990; Macdonald et al., 2003; Mifsud et al., 2008).

How T cell receptor recognizes MHC and peptide and how they play the vital roles in controlling diseases or inducing diseases attract popular interests(Zinkernagel & Doherty, 1974). As for alloreactivity reactions, most of the researches discussed the injury they induced against self--tissues(D'Orsogna et al., 2010; Montgomery et al., 2018; Stauss et al., 2004; Vionnet et al., 2020). We wondered if alloreactivity reactions could play good roles naturally or even artificially. Recently, A subset of T cells that cross-recognized the TL9 epitope bound by B*81:01 or B*42:01 alleles was identified in HIV-infected people(Ogunshola et al., 2018). And these cross-reactive T cells are correlated with the better outcome for HIV-infected patients, which showed the potential for clinical therapy. Why this alloreactivity happens and how it can be protective against HIV attracts our interests. We expressed and tested a cross--restricted TCR named T18A identified from HLA-B*8101 positive individuals. Theclonenamed '18A12' in the Ogunshola reference(Ogunshola et al., 2018), was HLA-B81/B42 dual-restricted and was confirmed to accommodate wildtype and escape variants of the TL9 epitope.

Although multiple HLA-B alleles can present the TL9 epitope, the frequency and pattern of TL9 epitope mutations are distinct, and have different effects on HIV-1 replication ability(Edwards et al., 2002; Frater et al., 2007; Leslie et al., 2006; Ntale et al., 2012). Several explanations were raised for the differential selection pressure exerted on HIV-1 by closely related HLA alleles, including various TCR clonotype usage, different TCR affinities resulting in different cross-recognition properties for TL9 variants(Geldmacher et al., 2009; Kløverpris, Leslie, & Goulder, 2015; Leslie et al., 2006), and the completely distinct interact surface presented by TL9 in HLA-B*81:01 and HLA-B*42:01(Kløverpris, Cole, et al., 2015). A phenomenon is suggested by these factors: there are different escape pathways of HIV-1 to adapt to different selection pressures when confronted with the $CD8^{+}$T cell response targeting the same epitope but restricted by different HLA molecules. At a population level, this may result in differential HLA-associated viral replication capacity and disease prognosis(Carlson et al., 2012).

In this study, we investigated the mechanism of the high-affinity $CD8^{+}$ T cell response to immunodominant HIV-1 epitope Gag-TL9 by reporting its TCR-pHLA ternary-complex structure. The cross-restriction structure of the same TCR showed that the T18A adopts very similar binding orientations although the conformation of the peptide Gag-TL9 are largely different when Gag-TL9 bound to its host-selecting B*81:01-TL9 and allogeneic B*42:01-TL9 molecules. Remarkably, the conformation of the B*42:01-presented TL9 peptide in complex with T18A is similar to the conformation of B*81:01-presented TL9 in complex with T18A. Thus, binding by TCR T18A selects for the B*81:01-bound conformation of the TL9 peptide. Another interesting feature of the complex is that CDR3β of T18A adapts a rare docking position over the conservative MHC surface. The CDR3β loop does not contact the peptide at all, instead of contacting with the MHC α2 helix. On the other hand, the CDR2β loop of T18A performs some contacts with the portion of the peptide that would normally be intensively contacted by CDR3β, which likely explains the T-cell receptor beta variables (TRBV) 12-3preference in dual-reactive TCRs(Leslie et al., 2006; Ogunshola et al., 2018). We summarized the featured sequenceof CDR3β and MHC α2 helix that interacted with each other. When back checked in various databases, these featured sequence pairs of CDR3β and HLA were repeatedly shown up in multiple other cases and diseases, which indicated a strong link between the two. For a long time, hot spot interactions between CDR2 and MHC or CDR1 and MHC were revealed while extremely diversified CDR3 was taken granted for recognizing peptide antigens. However, this CDR3β display the extraordinary features to represent an unclassical recognition pattern to recognize MHC only. The show up of this unclassical recognition pattern in long-time HIV controls and the structural basis of this complex provides unexpected insights for TCR cross-reactivity, alloreactivity and key points for controlling diseases such as HIV, a chronic virus with epitope mutating, by the immune system.

2. Materials and methods

2.1. Peptides

Peptides used in this paper, such as TL9 peptide (TPQDLNTML180-188) and the escape variant Q182S, Q182T, T186S, and Q182S/T186S TL9 peptide were synthesized at > 95% purity, were synthesized at GL Biochem corporation (shanghai, China).

2.2. Analysis on sequence of HIV-1 Gag TL9 epitope from subject studies

To clearly define the HLA-B*81:01 and B*42:01-mediated differential HIV-1 epitope evolution, we collected the viral sequencing profiles from published subject studies restricted to Gag TL9 epitope from 2007 to present. More than 20 literatures were obtained. Due to the different scope of statistics from various studies, however, we summarized all the data and divided it into two categories: a) A total of 584 HIV-1 infected individuals with clearly identified mutant residues at TL9 epitope. b). All data were combined together, a total of 3092 HIV-1 infected persons, but with less information about the mutated residue of TL9. The data was analyzed and visualized in Fig. S5 and table S4.

2.3. Protein expression and purification

The B*81:01/B*42:01 dual-reactive T18A TCR, mono-reactive B*42:01-restricted T7A TCR, and mono-reactive B*81:01-restricted T11A TCR were bacterially expressed as previously described(Cole et al., 2006, 2008; Hellman et al., 2016). The soluble HLA-B*42:01-TL9 and HLA-TL9-variants forms were also produced from bacterially expressed inclusion bodies. In brief, the α-chain and β-chain of TCR, the heavy chain and β2m of HLA were expressed in *Escherichia coli* BL21 (DE3) cells as inclusion bodies. The inclusion bodies were washed three times, dissolved in 8 M urea, and then mixed thoroughly in cold refolding buffer at a certain ratio. During TCR renaturation, α and β chains were added 1:1 to a buffer containing 50 mM Tris (pH 8.3), 2 mM EDTA, 2.5 M urea, 0.5 mM oxidized glutathione, and 5 mM reduced glutathione. During pMHC renaturation, HLA-B*81:01 or B*42:01 heavy chain and β2m were added 1:1 to another buffer containing 100 mM Tris-HCL (pH 8.3), 2 mM EDTA, 400 mM L-arginine-HCl, 0.5 mM oxidized glutathione, and 5 mM reduced glutathione. Peptides were dissolved in DMSO and then injected exceeding 5-fold the molar of MHC into refolding buffer. TCR and pMHC complexes were slowly stirred in refolding buffer for 74 h and 48h at 4 °C, respectively. TCR and pMHC proteins after renaturation were

dialyzed and further purified by ion exchange chromatography (HiTrap Q HP; Mono Q; GE Healthcare) and size-exclusion (Superdex 200; GE Healthcare) as described previously(Petersen et al., 2014; Pieper et al., 2018). The purified protein was quantitative to 10 mg/ml in buffer 10 mM Tris (pH 8.0) for crystallization.

2.4. Surface plasmon resonance

The SPR assays were performed as described previously(Blevins & Baker, 2017; Piepenbrink et al., 2009; Riley et al., 2018). Briefly, the protein was biotinylated in PBS for 1h at room temperature. The T18A TCR was immobilized on the streptavidin-coated flow-cell surface of a SA sensor chip and the pMHC complexes with concentration ranges of 0.5–250 μM were used as analyte. and the equilibrium affinities were measured on the Octet QKe system (ForteBio) at 25 °C in 10 mM HEPES, pH 7.4, 500 mM NaCl, 1%BSA, and 0.02%Tween20. The Kd was determined by the fitting of a single-ligand binding model.

2.5. Crystallization

Protein crystals of TCR-pMHC complexes were performed using the sitting-drop vapor diffusion method at 20 °C. The T18A in complex with HLA B*42:01 and Gag TL9 was crystallized in the buffer of 0.1 M SPG, 25% w/v PEG 1500, pH 7.0. Crystals were soaked in 20% glycerol/80% mother liquor for a few seconds and frozen into liquid nitrogen. Data were collected at the Shanghai Synchrotron Radiation Facility (Shanghai, China) on beam line BL17U1/BL18U1/BL19U1 and processed with HKL2000. The structures were determined by molecular replacement method using PHENIX.phaser. Refinement was done with PHENIX.refine and Coot.

2.6. Bioinformatics analysis

The public IEDB (http://www.iedb.org/), TCGA (https://portal.gdc.cancer.gov/) and GTEx v6 (https://gtexportal.org/home/) datasets and 10 x Genomics Document Library (https://www.10xgenomics.com/resources/application-notes/) were used for bioinformatics analysis.

2.7. Code availability

The atomic coordinates and structure factors were deposited in the Protein Data Bank, www.pdb.org (PDB ID codes 7DZN).

3. Results

3.1. Identical TCR docking modes of the T18A T cell receptor complexed to HLA-B*81:01/TPQDLNTML and HLA-B*42:01/TPQDLNTML

To critically examine why T18A TCR can creatively bind to distinct antigen-presenting surfaces in different HLA contexts, we previously obtained the structure of T18A and TPQDLNTML in the B*81:01 complex (PDB: 7DZM)(Liu et al., 2022) and determined the structure of T18A and TPQDLNTML in B*42:01 complex in this study. The statistics of the T18A TCR/B42/TL9 crystals were described (Table S1), and the structures of the ternary complexes were shown (Fig. 1A). The T18A TCR accommodates both peptide-HLA complexes in a similar traditional diagonal manner, with a total buried surface area (BSA)(Chothia & Lesk, 1987) of 1613.1 $Å^2$ in B*42:01 background, which fell within the range of known BSA(Rossjohn et al., 2015). The relative contact footprint of the CDR loops at the TCR-pHLA interface was shown in Fig. 1B.

Previous studies reported the enrichment of TRBV12-3 gene usage among TL9-specific T cells(Geldmacher et al., 2009; Leslie et al., 2006). Throughout TCR sequencing of the dual-reactive T cells from B*81:01 and B*42:01 expressing individuals, the dual-reactive TCR clone encoded TRBV12–3, T18A, was identified(Ogunshola et al., 2018). Not all of the complementarity determining region (CDR) loops contributed equally to the interaction, the TRBV12-3 β chain contributed 61.5% in B42 background to the interface of complexes. Moreover, CDR2β and CDR3β loops were the major contributors (34% and 21% BSA in B42 background) to this interaction (Fig. 1C). At the interface of the TCR-pHLA complex, hydrogen bonds and salt bridge (CDR3β-D100 with B*42:01 R153) was observed (Table S2). TL9 peptide contribute 15% to the BSA in the complex. The CDR3α and CDR2β of the T18A dominated the interaction between TCR and peptide (CDR3α 50%, CDR2β 41% in B4201) (Fig. 1D).

Interestingly, most TCRs used CDR3α and CDR3β to accommodate the various epitopes, but the role of CDR3β was different in the TCR T18A/B4201/TL9 complex. This unusual TCR CDR3β usage was observed previously in TCR T18A/B8101/TL9 complex we reported (PDB: 7DZM)(Liu et al., 2022). To conclude, the structural determination of this study revealed that TCR T18A adopts essentially identical conformation at the peptide-MHC interface across B*42:01 and B*81:01 restriction. Accordingly, this TCR cross-restriction event was not attributable to differential TCR docking mode across the distinct HLA-B alleles.

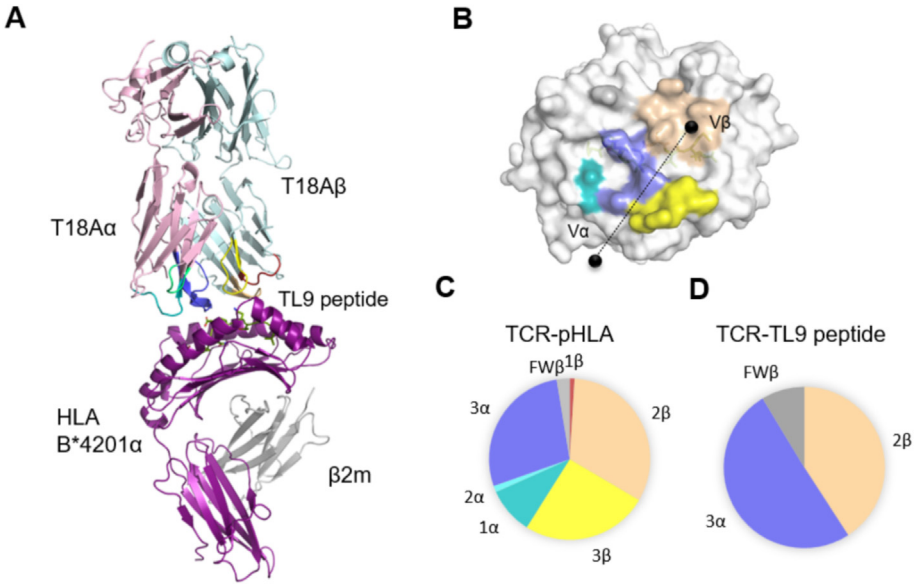

Fig. 1. The overview of crystal structure of TCR T18A/B42/TL9 complex. (**A**) The T18A TCR (T18Aα in pale pink, T18Aβ in pale cyan) recognize TL9 epitope presented by HLA-B4201. (**B**) The footprint of T18A TCR on the surface of each HLA-B42-TL9 complex. (**C-D**) Pie charts represent the contribution of TCR segments toward the pHLA complex. The CDR1α, CDR2α, and CDR3α loops are shown in teal, lime-green, and blue, whereas the CDR1β, CDR2β, and CDR3β loops are shown in firebrick, light orange, and yellow, respectively.

3.2. T18A TCR accommodation selects for the B81-bound conformation of the TL9 peptide

Previous study confirmed that the TL9 residues exposed to T cells were different in the epitope presentation with B8101 and B4201 (PDB: 4U1I, 4U1J)(Kløverpris, Cole, et al., 2015). A conformational switch of the TL9 peptide was confirmed (Fig. S1A), and residues 5–7 were in distinct orientations in each peptide-presentation platform. This switch induced a differentiated presentation of TL9 by B8101 compared to B4201 and B0702. In their study, Kloverpris et al. concluded that identical peptide (TL9-Gag, RM9-Nef) presented by closely related HLA I alleles (B*8101, B*4201, B*4202, and B*0702) are recognized as distinct epitopes, which underpinned differential HIV-1 escape seen in B81- versus B42- positive patients(Kløverpris, Cole, et al., 2015).Next, the configuration change of TL9 peptide before and after TCR accommodation was investigated. Firstly, the backbone of TL9 peptide from four complexes (B8101-TL9, B4201-TL9, B8101-TL9-T18A and B4201-TL9-T18A) were overlapped (Fig. 2A), and the TL9 backbone of TCR free, B4201 presentation was distinct compared to other three conformations. Secondly, the side chains of the TL9 peptide were overlapped in turns. HIV Gag-TL9 epitope exhibits distinct conformations (r.m.s. deviation of 2.15 Å) when presented by B8101 versus by B4201 (Fig. 2B, Fig. S1B, Fig. S2), but adapts essentially identical conformation (r.m.s. deviation of 0.25 Å) after TCR binding in the context B8101 and B4201 (Fig. 2C).

Under the B*42:01 restriction, the electron density showed that the central part of TPQDLNTML had a 'conformational switch' compared to its conformation in the free pMHC (Fig. 2D). The side chain of leucine at P5 (P5L) turned down with a movement of about 5.2 Å, and its peptide backbone was pressed toward the antigen-binding cleft. At the same time, anchor residue P6N was flipping by 112°, becoming solvent exposed and was involved in CDR2β interactions. On the contrary, solvent exposed P7T shifted towards the base of the groove by 4.3 Å and

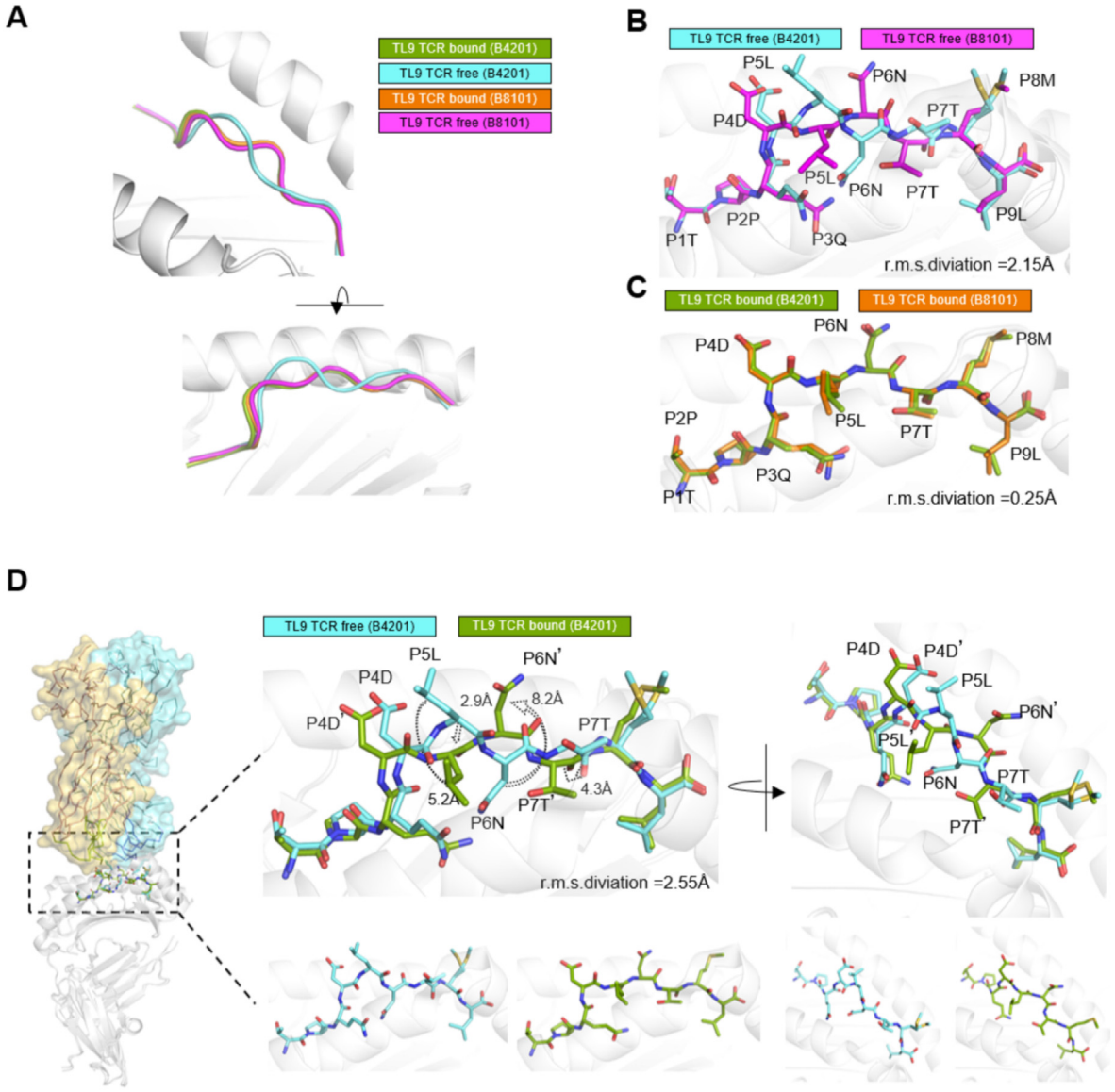

Fig. 2. Binding by TCR T18A induces a flip of TL9 peptide and selects the B81-bound conformation of the TL9 epitope. (**A**) The molecular switch of TL9 peptide seems due to TCR binding make its conformation closer to its B81-restricted conformation. (**B**) HIV Gag-TL9 epitope exhibits distinct conformations when presented by B8101 (PDB: 4U1I) versus B4201 (PDB: 4U1J). (**C**) TL9 peptide adapts essentially identical conformation after TCR binding when presented by B8101 (PDB:7DZM) and B4201. (**D**) The diagram of TCR-binding-induced TL9 flip in the B4201 presentation. The side chain and backbone of P5L is pressed into the bind groove for about 5 Å, the solvent exposed P7T is also press to the bind groove for 4.3 Å. The buried residue P6N shifts upwards by 8.2 Å and contact to the CDR2β of T18A.

acted as a secondary anchor residue. The 'molecular-switch' resulted in a less bulged conformation of TL9 peptide. From a structural perspective, TCR forced the side chain of the most exposed P5L and backbone of P4–P5 to embed towards the antigen-binding cleft, and popped out the asparagine up and out of the binding groove. Remarkably change in peptide configuration was reflected at r.m.s. deviation of 2.55 Å when the bound and free HLA-B*42:01 TL9-binding domains were superimposed.

Collectively, in the B42 context, TCR binding induced the conformational switch of the TL9 and made its backbone much closer to, the conformation of TL9 of B81 restriction. Thus, binding by T18A selected for the B81-bound conformation of the TL9 epitope.

3.3. HLA-B cross-restriction is underpinned by an induced-fit mechanism

It was intriguing to investigate how the B42-bound conformation of TL9 peptide, was induced and changed to B81-bound conformation. The superimpose of unbound and bound TL9/HLA-B4201 complexes confirmed that the clashes with CDR3α loop drive the conformational switch of the peptide (Fig. 3, left panel). Clashes on peptide involved the side chain of P4D and both backbone and side chain of P5L, which competed with Asn96 and Asn97 of CDR3α of TCR. The most serious clashing occurred between the side chain of P5L and the side chain of Asn97, which both occupied the same volume. As TCR and peptide ligands both owned certain extent of plasticity, we wondered why it was peptide itself to adapt to TCR accommodation but not in reverse. A net of hydrogen bonds was observed in the bottom end of CDR3α loop, which fixed the structure of CDR3α backbone (Fig. 3, right panel). Moreover, W149, K148, T145 and Y86 of HLA-B4201 formed a salt bridge and three hydrogen bonds with P9L and P8M at the TL9 C-terminus. The strong anchoring of C-terminal of the peptide limited the conformational switch in the middle of the peptide, rather than an extending of the peptide C-terminus. Accordingly, both the rigid CDR3α loop and C-terminal anchoring drive the structural rearrangement of the TL9 peptide, and the induced conformation of the peptide fit for TCR engagement.

3.4. The HLA-B polymorphism is accommodated by small TCR side chain rearrangement and favors the cross-restriction

Affinity measurements were then used to assess the strength of the cross-restriction event. Binding capacity of T18A TCR to different *p*-MHC molecules were measured by *in vitro* surface plasmon resonance (SPR). We previously reported that T18A could recognize the TL9 peptide presented by B*81:01 with a high affinity (K_D ~ 4.7 μM), and could effectively recognize some escape variants of TL9, such as 7s-TL9 (K_D ~ 31.4 μM)(Liu et al., 2022). Similarly, T18A was able to recognize TL9 peptides presented by B*42:01 with moderate affinity (Kd ~ 46.1 μM), as well as 7s-TL9 (K_D ~ 51.4 μM) (Fig. S3A), further supported the dual-reactivity of TCR T18A. SPR binding data for T18A TCR recognition of the wildtype (WT) and popular mutated TL9 presented by B8101 and B4201 were summarized in (Fig. S3B). Native-PAGE assays also confirmed the cross-restriction which was depicted by distinct migrations formed by T18A and B8101-TL9, B4201-TL9, respectively (Fig. S4). Thus, difference on binding affinity was seem for T18A TCR to B81-TL9 versus to B42-TL9, although the structures of complexes were quite similar.

In addition, we want to evaluate the influence of HLA polymorphism in the cross-restriction. HLA-B*81:01 and HLA-B*42:01 are two popular alleles in the African population, differ by 5 residues, of which 3 are located in the peptide-binding groove and may contribute to the interaction (Fig. 4A). The T18A TCR contacted 12 residues on HLA-B81 or B42: 8 from the α1 helix and 4 from the α2 helix. Of these contact points, T145 and W149 of HLA-B*42:01 had stronger interactions with TL9 peptide, which fixed the peptide C-terminus and contributed to the conformational adaptation of peptide upon T18A binding. However, E165 of HLA-B*81:01 formed hydrogen bonds with Asn96 of T18A CDR3α, which might contribute to a stronger TCR-MHC interaction than HLA-B*42:01.

Then we focused on the TCR landing surface aiming to find out the factors that might contribute to the differential affinity of the cross-restriction. Polymorphic residues, including S145α/T145α, L149α/W149α, and E165α/T165α, did not alter the binding register nor the conformation of the TL9 peptide. However, HLA-B81-TL9 and HLA-B42-TL9 are significantly different at the electrostatic surface potentials (Fig. 4B), due to the removal of the negative charge upon substitution of glutamic acid to threonine. Thus, amino acid polymorphism created difference in property of the TCR landing surface.

Here we aimed to find out how the cross-restricted TCR T18A accommodates the essential polymorphism created by E165α/T165α. The nearby residues of E165α/T165α were compared (Fig. 4C), and we found that TCR side chain was slightly rearranged. Asn96 of CDR3α interacted with E165 of HLA-B*8101, but the side chain T165 of B*4201 was shorter than E165 of B*8101 with a distance of about 5.2 Å so this H-bond was absent in B*4201 context. It was Asn32 of CDR1α formed hydrogen bond with A160 under the restriction of B*4201, as compensation. In B*8101 context, Asn32 of CDR1α was swinging towards the MHC α1 helix and forming H-bond with E165 via the help of water molecule. To evaluate to what extent the rearrangement happening, the CDR3 loops of T18A TCR of two structure complexes were superimposed (Fig. 4D). Although the configuration of TCR was almost similar, a small pivoting about 1 Å was observed mainly on CDRα loops but not CDRβ

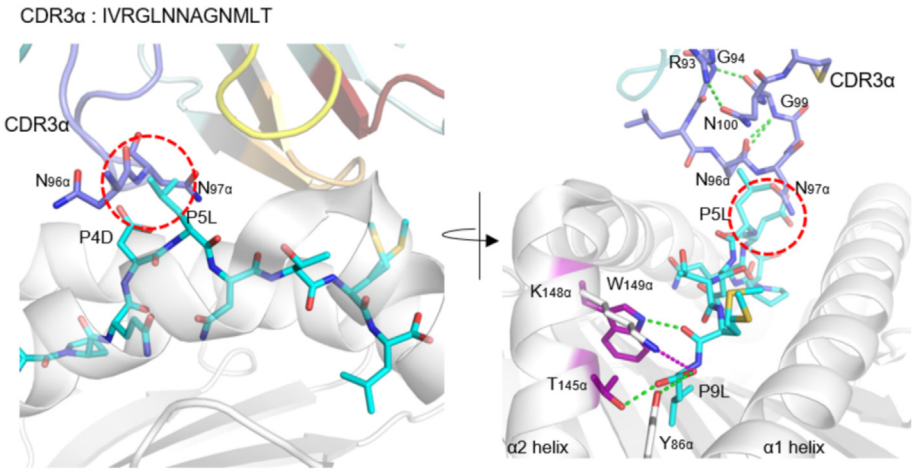

Fig. 3. The TL9 peptide presented by B42 changes its conformation to avoid steric clashes, and the amino acid polymorphisms between HLA-B42 and B81 alleles Left panel: Steric clashes between peptide (cyan) N-terminal P4D, P5L and Asn96, Asn97 of CDR3α (blue). Right panel: hydrogen bonds matrix increases CDR3α rigidity and drives the peptide to adapt TCR accommodation, instead of TCR to adapt to peptide. And the anchoring of peptide C terminus by W149α, K148α, T145α, and Y86α also contribute to conformational change in the TPQDLNTML peptide upon TCR involving. Hydrogen bonds are depicted by green dashed lines and the salt bridge is shown as purple dashed lines.

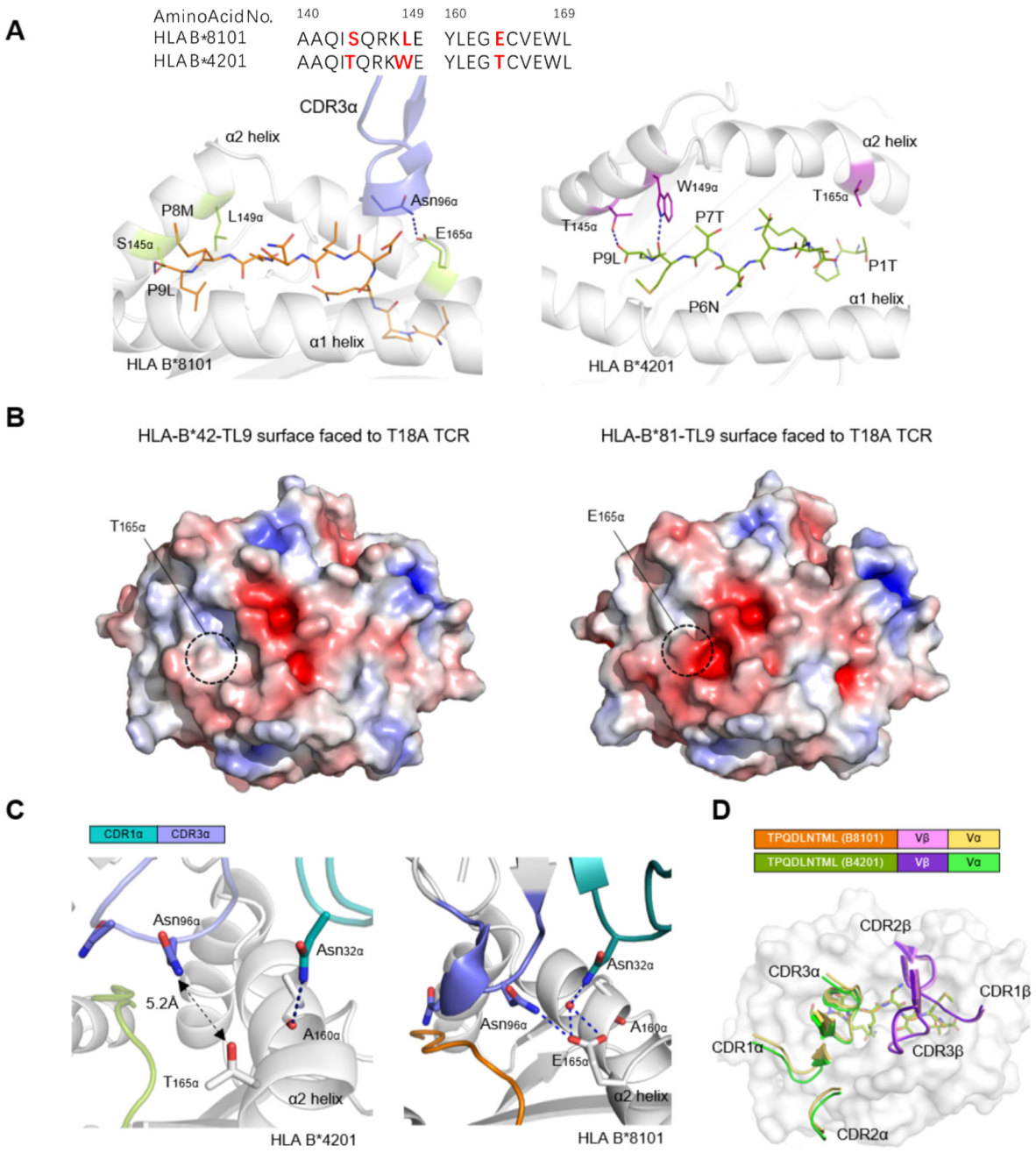

Fig. 4. The polymorphic HLA surfaces are recognized by TCR T18A through small TCR side chain rearrangement and different use of bridging water. (**A**) Sequence alignment of the two HLA-B alleles used in this study. Amino acid polymorphisms are shown in red. View of the HLA-B polymorphisms in the peptide binding groove. Compare to S145α, L149α in B8101, T145α, W149α in B4201 anchor the C terminus of peptide tighter than B8101 (PDB:7DZM) and contribute to conformation change of peptide flipping. The blue dashed lines represent hydrogen bonds. (**B**) Comparison shows the difference on the TCR landing surface of two alleles. The electrostatically colored surface of TL9-HLA-B8101 (PDB:7DZM) or -B4201 in complex with T18A binding are depicted, and the key polymorphic residues are labeled in dashed circle. Red color indicates the negatively charged and blue color represents positively charged residues. (**C**) Detailed view of the interactions nearby the polymorphic residue E165 or T165 in B81 (PDB:7DZM) or B42, respectively. The differential use of interfacial water molecule enables the small TCR side chain rearrangement. (**D**) Structural rearrangement of TCR CDRα loops occurred upon TCR binding to accommodate MHC alpha chain polymorphism.

loops. Accordingly, small TCR side chain rearrangement of T18A which occurred on CDRα loops accommodates the HLA-B polymorphism, thus also favoring the cross-restriction.

3.5. Unusual TCR CDR3β docking on MHC component underpins tolerance to peptide diversity

In both complexes, CDR3β loops were located above the α2 helix of the HLA protein, and were far away from the peptide side chains with a distance of about 9 Å, on average (Fig. 5A, Fig. S5). The CDR3β formed salt bridges between Asp100 and R153 of the HLA molecule, while Ile99 formed hydrogen bonds with R153 and A152 of the α2 helix. CDR3β of T18A formed strong contacts with the bulge of MHC α2 helix and was one of the main contributors of the TCR-pMHC interaction. The CDR3β was clearly with no interactions towards the peptide, which was unexpected and intriguing. As compensations, CDR2β shifted over the peptide antigen with less contacts.

Generally, T cell receptors display high diversities in CDR3 regions to

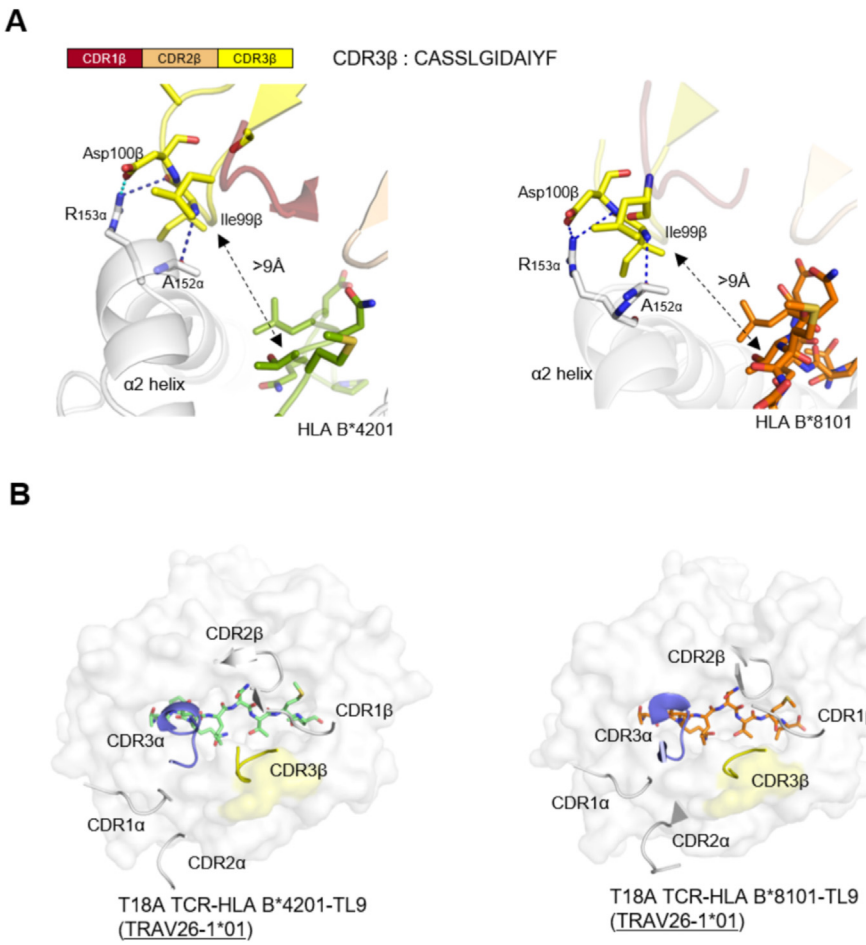

Fig. 5. The rare docking mode of T18A CDR3β which doesn't contact the peptide all. (**A**) CDR3β loops of both two structures of complex forms 1 salt bridge and 2 hydrogen bonds to HLA α2 helix, and are far away from the TL9 peptide in T18A TCR/B81/TL9 (PDB:7DZM) and T18A TCR/B42/TL9 complexes. The deep blue dashed lines represent hydrogen bonds and the cyan dashed lines represent salt bridges. (**B**) The foot print of T18A TCR CDR3β on B81-TL9 (PDB:7DZM) or B42-TL9 are colored in yellow. Peptide in each panel is shown in stick, CDR loops are shown in cartoon, and MHCs are shown in surface view.

contact varied antigen peptides while less diversified CDR1 and CDR2 loops mainly contact the less varied MHC molecules. In the docking of T18A TCR toward B8101 or B4201, however, CDR3β formed no contacts to the peptide but focused on the α2 helix of MHC (Fig. 5B). Thus, the role of CDR3β of T18A was unique, which did not contact the peptide instead of forming rigidly interactions with the MHC α2 helix. The classical and non-classical TCR CDR3β recognition was shown in cartoons (Fig. 6C).

The unusual CDR3β docking of T18A on MHC component and CDR2β over the peptide with less contacts might lead to the loose restriction of the peptide antigen and contribute to the cross-reactive property of T18A. This property of T18A extended its tolerance to mutated peptides and might be related to the delayed viral escape in the clinic(Ogunshola et al., 2018). The interacted featured sequence pairs of CDR3β and MHC were shown in Table 1A. The summary of the TCR atom-to-atom contacts with the two ligands (B81 and B42) was displayed in Table 1B. However, whether the interacted featured sequence was present in other diseases and performed an important role is not clear. We next screened the featured sequence pairs of CDR3β and MHC, one case in IEDB dataset and two cases in a public data set from a single-cell pMHC-based T-cell specificity experiment derived from 10 x Genomics Document Library (Fig. 6A). In the above three cases, TCR interacted with pMHC has been proved. We wondered whether the featured TCR sequence exists in healthy people. Then, we screened the featured CDR3β sequence in Genotype-Tissue Expression (GTEx v6) project dataset and no similar CDR3β sequence was observed in healthy people. However, the featured sequence pairs of CDR3β and MHC were observed in cervical cancer in TCGA dataset. And in Liu's research about COVID-19(Liu et al., 2021), there are three cases of COVID-19 patients contained the featured CDR3β and MHC sequence (Fig. 6B).

4. Discussion

The MHC-restricted recognition of presented epitopes by TCRs is an essential event in the adaptive immunity against pathogens and surveillance of cancer cells. It also plays a central role in multiple immunological disorders, including allergy, autoimmune disease, and alloreactivity responses followed by organ transplantation. Although in most of the complexes TCR binding to peptide-MHC in a similar orientation, the chemical property and shapes of these interaction interface are variable, and minor changes might have dramatic influence to biological response. The different structures represent various biological responses, such as positive selection in thymus, anti-viral immune response and alloreaction still need to be reported.

A population of dual-restricted T cells associated with lower plasma viral load following HIV-1 infection is identified by Brockman and Ndhlovu et al.(Ogunshola et al., 2018), but the mechanism of the TCR recognition and the cross-restriction event remain a mystery. Herein we solved the crystal structure of the human T18A TCR in complex with a HIV-1 immunodominant epitope p24-Gag-TL9 presented by B*42:01, and analyzed the cross-reactive structures of T18A TCR to both B*81:01-TL9 and allogeneic B*42:01-TL9 molecules. Given the findings that TCR affinity is directly related to cytotoxic capacity of the TCR-transduced T cells(Campillo-Davo & Flumens, 2020; Solouki & Huang, 2020; Spear et al., 2019), our results provide insight into the molecular mechanism that $CD8^+$ T cells expressing such TCRs will contribute to HIV control.

The TL9 epitope was previously shown to be presented by two closely related HLA alleles B8101 and B4201 in markedly different conformations that flip several of the TCR accessible residues, and it was indicated that this difference in MHC-bound epitope conformation is responsible

A

Data source	CDR3ß aa sequence	HLA name	HLA exists the similar aa sequence (p-HLA contacts with TCR)	Peptide	Peptide source
IEDB Database	CASLRLGIDQETQYF	B0801	AARVAEQ	RAKFKQLL	EBV
10 x Genomics Document Library	CASSLGIDIQYF	A0301	AAHEAEQ	KLGGALQAK	CMV
	CASSLGIDYEQY	A0301	AAHEAEQ	KLGGALQAK	CMV

B

Data source	Disease	CDR3ß aa sequence	HLA name	HLA aa sequence	% of contain "LGID"sequence in total CDR3ß sequence
TCGA Dataset	Cervical Cancer	CASSLGIDGGAF	A6801 B4001	AARVAEQ AARVAEQ	0.00836
Liu Y, *et al*, 2022[42]	COVID-19	CASSLGIDTQYF	A1101 B4001 C0801	AAHAAEQ AARVAEQ AARTAEQ	0.00206
	COVID-19	CATSDPLGIDNEQFF	A1101 B4001 B5801	AAHAAEQ AARVAEQ AARVAEQ	
	COVID-19	CASSLGIDQPQH	B3501	AARVAEQ	

C

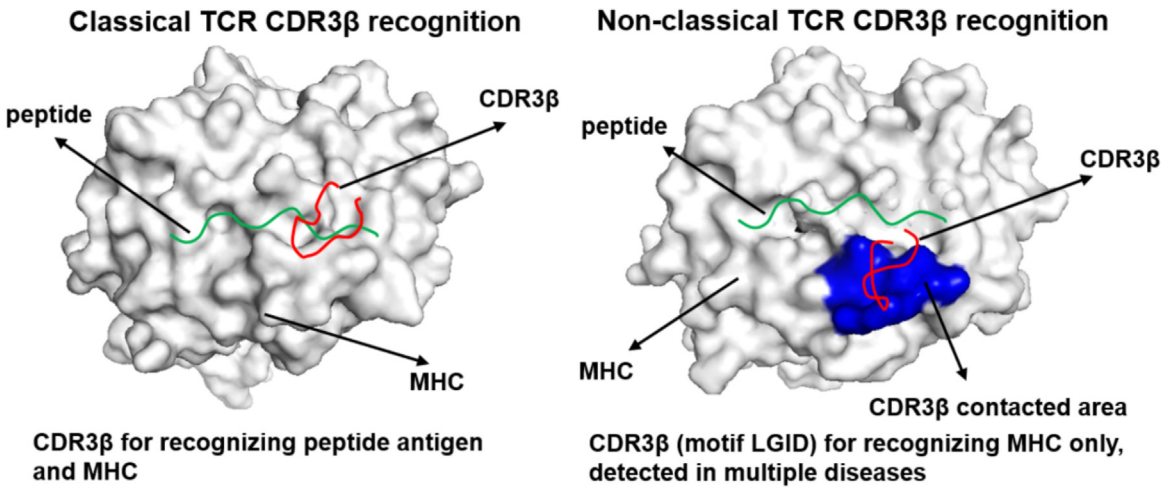

Fig. 6. Cartoons of non-classical TCR CDR3β recognition. (**A-B**) Special sequence are observed in other diseases. (**C**) Cartoons of TCR recognition.

for the differential viral control found in B81- versus B42-positive patients. In this study, we reported the TCR recognition structure of the TL9 epitope presented by B4201. Moreover, we also analyzed a cross-restriction structure of the T18A TCR in complex with TL9 presented by B8101 and by B4201. Notably, the conformation of the B81-presented TL9 epitope in complex with T18A is essentially identical to the conformation of B42-presented TL9 in complex with T18A, which is itself very similar to the B81-TL9 complex in the absence of TCR. Accordingly, binding by TCR T18A chooses the B81-bound conformation of the TL9 epitope. Thus, the central point of this cross-restriction is the TCR binding induces a molecular mimicry of the peptide conformation of TL9.

Interestingly, why the dual-reactive T cells are correlated with better control against HIV-1 infection, instead of the mono-reactive T cells. This could be explained by viral escaping on the immune-dominant TL9 epitope. Our results revealed that the TL9 peptide is flexible in the antigen-binding cleft, so besides attenuating epitope presentations, mutations on the TL9 could possibly challenge the effective TCR recognition by changing residues facing the TCRs. Considering TL9 peptide exposed distinct residues to T cells with B8101 or B4201, thus it is a great challenge for mono-reactive T cells to cope with diversified interaction surfaces. Thus, the escape mutations on the TL9 epitope might sometimes change the peptide conformation and escape the pre-existing effective T cells. However, this escape strategy could be blocked by cross-reactive T cells.

From a structural perspective, the absence of CDR3β in interactions toward peptides and intensive interactions of CDR3β toward MHC make the dual-reactive TCR T18A less specific but more versatile. Polymorphic alleles B8101 and B4201 do influence the conformation of the peptide, but T18A TCR overcomes this challenge. Unique CDR loop usage enables T18A to tolerate different initial conformations of the TL9 epitope. We have presented binding data from SPR assays showing that the T18A TCR is capable to recognize the mutated version of the TL9 peptide (Fig. S2), as well as results of a few single-reactive (ie. B42 or B81 restricted but not dual-reactive) TCR clones showing that they are unable to do so (Fig. S6

Table 1
Summary of contacts between the T18A TCR and Its ligands. (**A**) The interacted sequence between CDR3β and MHC. (**B–C**) TCR atom-to-atom contacts with Its ligands.

A

TCR name	CDR3β aa sequence	HLA name	HLA aa sequence contacts with CDR3β
T18A	CASSLGIDAIYF	B8101 B4201	AARVAEQ AARVAEQ

B

TCR CDR	Amino Acid	B81	TL9 peptide	Van der Waals interaction	Total	% of CDR domain in total TCR interaction
α1	Y34	6	–	1	6	6.1
α3	L95	4	1	26	34	34.7
	N96	1	7			
	N97	–	17			
	A98	–	4			
β2	N51	–	7	22	26	26.5
	I52	–	6			
	N53	2	–			
	V54	7	–			
	I56	1	3			
β3	L97	3	–	30	32	32.7
	G98	5	–			
	I99	18	–			
	D100	6	–			

C

TCR CDR	Amino Acid	B42	TL9 peptide	Van der Waals interaction	Total	% of CDR domain in total TCR interaction
α1	N32	3	–	7	9	9.4
α3	Y34	6	–			
	L95	6	1	26	30	31.3
	N96	–	5			
	N97	–	14			
	A98	–	4			
β2	N51	–	8	27	29	30.2
	N52	3	6			
	N53	1	–			
	V54	7	–			
	I56	1	3			
β3	L97	1	–	27	28	29.2
	G98	3	–			
	I99	21	–			
	D100	3				

and Table S3). SPR assays confirm that the affinity of dual-reactive T18A TCR for TL9-HLA, especially for mutated epitopes, was stronger than that of single-reactive TCR T11A and T7A. Besides defining antigen-specificity, the affinity of TCR to pHLA is directly correlated to the toxicity and proliferative capacity of TCR-transduced T cells, which further explains the clinical benefit of the presence of dual-reactive T cells.

Of note, the polymorphism at position 165 of MHC α2 helix (glutamic acid in B8101 but threonine in B4201) explains why the affinity of T18A against B8101-TL9 is higher than that of T18A against B4201-TL9, as H-bond is formed only between 165E and CDR3α. A stronger $CD8^+$ T immune response therefore produces greater selection pressure for HIV-1 in the B*81:01 population. Interestingly, HIV-1 sequence analysis based on HIV-1 infected patients(Currier et al., 2006; Dorrell et al., 2001; Kløverpris et al., 2012, 2015c; Payne et al., 2014) showed that the mutation frequency of TL9 epitope in the B*81:01 expressing individuals was significantly higher than that in the B*42:01 expressing individuals and the cohort without the above two alleles (Table S4). We previously showed that under the background HLA-B*81:01, the TL9 epitope preferred mutations were 3s-TL9 and 7s-TL9(Liu et al., 2022). In this study, we found in the context of HLA B*42:01, the mutations in the TL9 epitope focus on position 3, and the most preferred mutation was 3t-TL9 (Fig. S7). A molecular arm race between protective T cell response and HIV-1 mutation is suggested by these studies, the influence of host acquired immunity in genomic evolution of the HIV, therefore, might be underestimated.

Thecross-reactive TCR T18A has a unique docking mode of CDR3β, this unique CDR3β usage strengthens the peptide tolerance of T18A, and thus increasing the capability of TL9 escape variants. These features are consistent with the better control of viral replication and delayed viral escape in B8101 individuals. Supported by these clinical and structural evidence, the dual-reactive phenotype of $CD8^+$ T cells might be good biomarkers for viral control and with great clinical significance for immunotherapy. These featured sequence of CDR3β was also observed in multiple other diseases with the special matching MHC sequence, such as EBV, CMV, cervical cancer and COVID-19.

In general, this study illustrated the unconventional recognition of this dual-reactive TCR clonotypes for long-time HIV controls. It also shed lights to our understanding of the cross-reactivity for multiple peptide mutants and the alloreactivity for different HLA alleles at the same time by one TCR. This dual-reactive TCR clonotypes are tolerant of antigen mutation and might be beneficial for the development of T cell vaccines and TCR-T cell therapy against important human diseases especially with epitope mutating, such as HIV or cancers.

Ethics approval

Not applicable. No patients are involved in this study. The clinical data are cited and summarized from published papers.

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Author contributions

LY and YSC conceived and designed experiments. JL and YL wrote the original manuscript. JL, YL and DS performed the research and analyzed the final data. JL, DS, YL, BWJ, XY, PCW, YY, JZ, PC and HJW contributed to data analysis. CP edited the language of the article. LY and YSC supervised the study and all authors contributed to revisions.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed in the study are available from the corresponding author on reasonable request.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.cellin.2022.100076.

References

Allen, P. M. (1994). Peptides in positive and negative selection: A delicate balance. *Cell, 76*, 593–596.

Blevins, S. J., & Baker, B. M. (2017). Using global analysis to extend the accuracy and precision of binding measurements with T cell receptors and their peptide/MHC ligands. *Frontiers in Molecular Biosciences, 4*, 2.

Campillo-Davo, D., & Flumens, D. (2020). The quest for the best: How TCR affinity, avidity, and functional avidity affect TCR-engineered T-cell antitumor responses. *Cells, 9*.

Carlson, J. M., Listgarten, J., Pfeifer, N., Tan, V., Kadie, C., Walker, B. D., Ndung'u, T., Shapiro, R., Frater, J., Brumme, Z. L., et al. (2012). Widespread impact of HLA restriction on immune control and escape pathways of HIV-1. *Journal of Virology, 86*, 5230–5243.

Chothia, C., & Lesk, A. M. (1987). Canonical structures for the hypervariable regions of immunoglobulins. *Journal of Molecular Biology, 196*, 901–917.

Cole, D. K., Dunn, S. M., Sami, M., Boulter, J. M., Jakobsen, B. K., & Sewell, A. K. (2008). T cell receptor engagement of peptide-major histocompatibility complex class I does not modify CD8 binding. *Molecular Immunology, 45*, 2700–2709.

Cole, D. K., Rizkallah, P. J., Gao, F., Watson, N. I., Boulter, J. M., Bell, J. I., Sami, M., Gao, G. F., & Jakobsen, B. K. (2006). Crystal structure of HLA-A*2402 complexed with a telomerase peptide. *European Journal of Immunology, 36*, 170–179.

Colf, L. A., Bankovich, A. J., Hanick, N. A., Bowerman, N. A., Jones, L. L., Kranz, D. M., & Garcia, K. C. (2007). How a single T cell receptor recognizes both self and foreign MHC. *Cell, 129*, 135–146.

Currier, J. R., Visawapoka, U., Tovanabutra, S., Mason, C. J., Birx, D. L., McCutchan, F. E., & Cox, J. H. (2006). CTL epitope distribution patterns in the gag and nef proteins of HIV-1 from subtype A infected subjects in Kenya: Use of multiple peptide sets increases the detectable breadth of the CTL response. *BMC Immunology, 7*, 8.

D'Orsogna, L. J., Roelen, D. L., Doxiadis, , II, & Claas, F. H. (2010). Alloreactivity from human viral specific memory T-cells. *Transplant Immunology, 23*, 149–155.

Dorrell, L., Willcox, B. E., Jones, E. Y., Gillespie, G., Njai, H., Sabally, S., Jaye, A., DeGleria, K., Rostron, T., Lepin, E., et al. (2001). Cytotoxic T lymphocytes recognize structurally diverse, clade-specific and cross-reactive peptides in human immunodeficiency virus type-1 gag through HLA-B53. *European Journal of Immunology, 31*, 1747–1756.

Doxiadis, , II, Smits, J. M., Schreuder, G. M., Persijn, G. G., van Houwelingen, H. C., van Rood, J. J., & Claas, F. H. (1996). Association between specific HLA combinations and probability of kidney allograft loss: The taboo concept. *Lancet (London, England), 348*, 850–853.

Edwards, B. H., Bansal, A., Sabbaj, S., Bakari, J., Mulligan, M. J., & Goepfert, P. A. (2002). Magnitude of functional CD8+ T-cell responses to the gag protein of human immunodeficiency virus type 1 correlates inversely with viral load in plasma. *Journal of Virology, 76*, 2298–2305.

Felix, N. J., & Allen, P. M. (2007). Specificity of T-cell alloreactivity. *Nature Reviews Immunology, 7*, 942–953.

Fleischhauer, K., Kernan, N. A., O'Reilly, R. J., Dupont, B., & Yang, S. Y. (1990). Bone marrow-allograft rejection by T lymphocytes recognizing a single amino acid difference in HLA-B44. *New England Journal of Medicine, 323*, 1818–1822.

Frater, A. J., Brown, H., Oxenius, A., Günthard, H. F., Hirschel, B., Robinson, N., Leslie, A. J., Payne, R., Crawford, H., Prendergast, A., et al. (2007). Effective T-cell responses select human immunodeficiency virus mutants and slow disease progression. *Journal of Virology, 81*, 6742–6751.

Galperin, M., & Farenc, C. (2018). CD4(+) T cell-mediated HLA class II cross-restriction in HIV controllers. *Science immunology, 3*.

Geldmacher, C., Metzler, I. S., Tovanabutra, S., Asher, T. E., Gostick, E., Ambrozak, D. R., Petrovas, C., Schuetz, A., Ngwenyama, N., Kijak, G., et al. (2009). Minor viral and host genetic polymorphisms can dramatically impact the biologic outcome of an epitope-specific CD8 T-cell response. *Blood, 114*, 1553–1562.

Hawke, S., Matsuo, H., Nicolle, M., Wordsworth, P., Corlett, L., Spack, E., Deshpande, S., Driscoll, P. C., & Willcox, N. (1999). Cross-restriction of a T cell clone to HLA-DR alleles associated with rheumatoid arthritis: Clues to arthritogenic peptide motifs. *Arthritis & Rheumatism, 42*, 1040–1050.

Hellman, L. M., Yin, L., Wang, Y., Blevins, S. J., Riley, T. P., Belden, O. S., Spear, T. T., Nishimura, M. I., Stern, L. J., & Baker, B. M. (2016). Differential scanning fluorimetry based assessments of the thermal and kinetic stability of peptide-MHC complexes. *Journal of Immunological Methods, 432*, 95–101.

Hennecke, J., & Wiley, D. C. (2002). Structure of a complex of the human alpha/beta 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. *Journal of Experimental Medicine, 195*, 571–581.

Kawase, T., Morishima, Y., Matsuo, K., Kashiwase, K., Inoko, H., Saji, H., Kato, S., Juji, T., Kodera, Y., & Sasazuki, T. (2007). High-risk HLA allele mismatch combinations responsible for severe acute graft-versus-host disease and implication for its molecular mechanism. *Blood, 110*, 2235–2241.

Kisielow, P., & von Boehmer, H. (1991). Kinetics of negative and positive selection in the thymus. *Advances in Experimental Medicine & Biology, 292*, 31–42.

Kløverpris, H. N., Cole, D. K., Fuller, A., Carlson, J., Beck, K., Schauenburg, A. J., Rizkallah, P. J., Buus, S., Sewell, A. K., & Goulder, P. (2015a). A molecular switch in immunodominant HIV-1-specific CD8 T-cell epitopes shapes differential HLA-restricted escape. *Retrovirology, 12*, 20.

Kløverpris, H. N., Harndahl, M., Leslie, A. J., Carlson, J. M., Ismail, N., van der Stok, M., Huang, K. H., Chen, F., Riddell, L., Steyn, D., et al. (2012). HIV control through a single nucleotide on the HLA-B locus. *Journal of Virology, 86*, 11493–11500.

Kløverpris, H. N., Leslie, A., & Goulder, P. (2015b). Role of HLA adaptation in HIV evolution. *Frontiers in Immunology, 6*, 665.

Kløverpris, H. N., McGregor, R., McLaren, J. E., Ladell, K., Harndahl, M., Stryhn, A., Carlson, J. M., Koofhethile, C., Gerritsen, B., Keşmir, C., et al. (2015c). CD8+ TCR bias and immunodominance in HIV-1 infection. *The Journal of Immunology, 194*, 5329–5345. Baltimore, Md : 1950.

Leslie, A., Price, D. A., Mkhize, P., Bishop, K., Rathod, A., Day, C., Crawford, H., Honeyborne, I., Asher, T. E., Luzzi, G., et al. (2006). Differential selection pressure exerted on HIV by CTL targeting identical epitopes but restricted by distinct HLA alleles from the same HLA supertype. *The Journal of Immunology, 177*, 4699–4708. Baltimore, Md : 1950.

Liu, Y., Lei, J., San, D., Yang, Y., Paek, C., Xia, Z., Chen, Y., & Yin, L. (2022). Structural basis for unusual TCR CDR3β usage against an immunodominant HIV-1 gag protein peptide restricted to an HLA-B*81:01 molecule. *Frontiers in Immunology, 13*, Article 822210.

Liu, Y., Wu, Y., Liu, B., Zhang, Y., San, D., Chen, Y., Zhou, Y., Yu, L., Zeng, H., Zhou, Y., et al. (2021). Biomarkers and immune repertoire metrics identified by peripheral blood transcriptomic sequencing reveal the pathogenesis of COVID-19. *Frontiers in Immunology, 12*, Article 677025.

Macdonald, W. A., Purcell, A. W., Mifsud, N. A., Ely, L. K., Williams, D. S., Chang, L., Gorman, J. J., Clements, C. S., Kjer-Nielsen, L., Koelle, D. M., et al. (2003). A naturally selected dimorphism within the HLA-B44 supertype alters class I structure, peptide repertoire, and T cell recognition. *Journal of Experimental Medicine, 198*, 679–691.

Marusić-Galesić, S., & Pavelić, K. (1990). Dynamics of positive and negative selection in the thymus: Review and hypothesis. *Immunology Letters, 24*, 149–154.

Mehrotra, A., Leventhal, J., Purroy, C., & Cravedi, P. (2015). Monitoring T cell alloreactivity. *Transplantation Reviews, 29*, 53–59.

Mifsud, N. A., Purcell, A. W., Chen, W., Holdsworth, R., Tait, B. D., & McCluskey, J. (2008). Immunodominance hierarchies and gender bias in direct T(CD8)-cell alloreactivity. *American Journal of Transplantation : Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons, 8*, 121–132.

Montgomery, R. A., Tatapudi, V. S., Leffell, M. S., & Zachary, A. A. (2018). HLA in transplantation. *Nature Reviews Nephrology, 14*, 558–570.

Mukhopadhyay, M., Galperin, M., Patgaonkar, M., & Vasan, S. (2017). DNA vaccination by electroporation amplifies broadly cross-restricted public TCR clonotypes shared with HIV controllers. *The Journal of Immunology, 199*, 3437–3452. Baltimore, Md : 1950.

Nagy, Z. A. (2012). Alloreactivity: An old puzzle revisited. *Scandinavian Journal of Immunology, 75*, 463–470.

Ntale, R. S., Chopera, D. R., Ngandu, N. K., Assis de Rosa, D., Zembe, L., Gamieldien, H., Mlotshwa, M., Werner, L., Woodman, Z., Mlisana, K., et al. (2012). Temporal association of HLA-B*81:01- and HLA-B*39:10-mediated HIV-1 p24 sequence evolution with disease progression. *Journal of Virology, 86*, 12013–12024.

Ogunshola, F., Anmole, G., Miller, R. L., Goering, E., Nkosi, T., Muema, D., Mann, J., Ismail, N., Chopera, D., Ndung'u, T., et al. (2018). Dual HLA B*42 and B*81-reactive T cell receptors recognize more diverse HIV-1 Gag escape variants. *Nature Communications, 9*, 5023.

Payne, R., Muenchhoff, M., Mann, J., Roberts, H. E., Matthews, P., Adland, E., Hempenstall, A., Huang, K. H., Brockman, M., Brumme, Z., et al. (2014). Impact of HLA-driven HIV adaptation on virulence in populations of high HIV seroprevalence. *Proceedings of the National Academy of Sciences of the United States of America, 111*, E5393–E5400.

Petersen, J., Montserrat, V., Mujico, J. R., Loh, K. L., Beringer, D. X., van Lummel, M., Thompson, A., Mearin, M. L., Schweizer, J., Kooy-Winkelaar, Y., et al. (2014). T-cell receptor recognition of HLA-DQ2-gliadin complexes associated with celiac disease. *Nature Structural & Molecular Biology, 21*, 480–488.

Piepenbrink, K. H., Gloor, B. E., Armstrong, K. M., & Baker, B. M. (2009). Methods for quantifying T cell receptor binding affinities and thermodynamics. *Methods in Enzymology, 466*, 359–381.

Pieper, J., Dubnovitsky, A., Gerstner, C., James, E. A., Rieck, M., Kozhukh, G., Tandre, K., Pellegrino, S., Gebe, J. A., Rönnblom, L., et al. (2018). Memory T cells specific to citrullinated α-enolase are enriched in the rheumatic joint. *Journal of Autoimmunity, 92*, 47–56.

Riley, T. P., Hellman, L. M., Gee, M. H., Mendoza, J. L., Alonso, J. A., Foley, K. C., Nishimura, M. I., Vander Kooi, C. W., Garcia, K. C., & Baker, B. M. (2018). *T cell receptor cross-reactivity expanded by dramatic peptide-MHC adaptability, 14*, 934–942.

Rossjohn, J., Gras, S., Miles, J. J., Turner, S. J., Godfrey, D. I., & McCluskey, J. (2015). T cell antigen receptor recognition of antigen-presenting molecules. *Annual Review of Immunology, 33*, 169–200.

Saito, T., & Watanabe, N. (1998). Positive and negative thymocyte selection. *Critical Reviews in Immunology, 18*, 359–370.

Sherman, L. A., & Chattopadhyay, S. (1993). The molecular basis of allorecognition. *Annual Review of Immunology, 11*, 385–402.

Sicard, A., Kanitakis, J., Dubois, V., Morelon, E., & Thaunat, O. (2020). Humoral alloreactivity in VCA recipients: Should we learn from our experience? *Transplantation, 104*, 2003–2010.

Solouki, S., & Huang, W. (2020). TCR signal strength and antigen affinity regulate CD8(+) memory T cells. *The Journal of Immunology, 205*, 1217–1227. Baltimore, Md : 1950.

Spear, T. T., Evavold, B. D., Baker, B. M., & Nishimura, M. I. (2019). Understanding TCR affinity, antigen specificity, and cross-reactivity to improve TCR gene-modified T cells for cancer immunotherapy. *Cancer Immunology, Immunotherapy, 68*, 1881–1889. CII.

Starr, T. K., Jameson, S. C., & Hogquist, K. A. (2003). Positive and negative selection of T cells. *Annual Review of Immunology, 21*, 139–176.

Stauss, H. J., Xue, S., Gillmore, R., Gao, L., Bendle, G., Holler, A., Downs, A. M., & Morris, E. (2004). Exploiting alloreactivity for tumour immunotherapy. *Vox Sanguinis, 87*(Suppl 2), 227–229.

Vionnet, J., Sempoux, C., Pascual, M., Sánchez-Fueyo, A., & Colmenero, J. (2020). Donor-specific antibodies in liver transplantation. *Gastroenterología y Hepatología, 43*, 34–45.

Vollmer, J., Weltzien, H. U., Gamerdinger, K., Lang, S., Choleva, Y., & Moulon, C. (2000). Antigen contacts by Ni-reactive TCR: Typical alphass chain cooperation versus alpha chain-dominated specificity. *International Immunology, 12*, 1723–1731.

Yousef, S., Planas, R., Chakroun, K., Hoffmeister-Ullerich, S., Binder, T. M., Eiermann, T. H., Martin, R., & Sospedra, M. (2012). TCR bias and HLA cross-restriction are strategies of human brain-infiltrating JC virus-specific CD4+ T cells during viral infection. *The Journal of Immunology, 189*, 3618–3630. Baltimore, Md : 1950.

Zinkernagel, R. M., & Doherty, P. C. (1974). Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. *Nature, 248*, 701–702.